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GERMINATION OF HAWAIIAN RANGE GRASS SEEDS

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ABSTRACT

Poor germination resulting from dormancy of seeds has been a major problem in use of many desirable species of Hawaiian range grasses.

Dormancy in seeds is the result of one or more of the following conditions: (1) presence of enclosing structures that hinder maximum expansion of the seed; (2) presence of structures that interfere with exchange of gases; (3) dormancy of the embryo itself; (4) need within the seed for stimulators of respiratory and nutritive activities; (5) presence of inhibitors produced by the seed hulls; (6) immaturity of embryos; (7) inability of seed to absorb water; (8) secondary dormancy.

The investigations reported in this bulletin determined the causes of dormancy operating in 10 species of Hawaiian range grasses. Methods of stimulating germination were developed. Of the causes of dormancy listed above, the first five were found to operate in the species studied.

Conditions stimulating dormant seeds to germinate were found to include soaking in water, cutting of the seed coat, acid scarification, mechanical scarification, subjection to alternating temperatures, removal of hulls, soaking in potassium nitrate and in ammonium thiocyanate, and after-ripening at warm temperatures.

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INTRODUCTION

Failure of germination of seeds[1] of range grasses grown in Hawaii has been a serious problem to ranchers and others interested in grass propagation. Because of their low germination, many species (particularly those of recent introduction which are not yet widely spread within the Territory) are being propagated vegetatively by crown divisions and stem cuttings. Propagation by seeds—if good germination can be obtained—has its advantages over vegetative propagation.

Unfortunately seeds of many of the desirable species do not germinate well under ordinary Hawaiian conditions. It was with the view of developing means to induce the germination of these species that the investigations reported here were initiated some years ago. The objectives were twofold:

(1) To develop methods which may be used in the field.
(2) To develop or find methods which the seed analyst may use to determine potential germination percentages of grass seeds.

Review of Literature

As the literature on seed germination is voluminous, only those references dealing with forage grass seeds will be reviewed here.

Perhaps the simplest type of seed treatment to induce germination of seeds is soaking the dormant seed in water before planting. Thus Wenger (63) found that soaking the burs of buffalo grass in tap water for 2 to 4 days, followed by a thorough drying at room temperature, increased the germination from 7.0 percent to 43.6 percent. Griswold (26), however, studying the effect of alternate moistening and drying on germination of seeds of 42 species of western range plants, found that the effects obtained were variable. In some species germination was hastened and increased, in some it was retarded and decreased, and in others, it was unaffected by the alternate moistening and drying treatment.

Oxygen supply plays an important role in the germination of seeds. Using the seed of Bermuda grass, Morinaga (37) obtained excellent germination

[1] The word "seed" as used in this bulletin refers to the caryopsis or grain, and the words "seed coat" to the fused pericarp and seed coat.

under reduced oxygen pressure. In air diluted with 40 to 60 percent H_2 or N_2 by volume, the germination was approximately 90 percent after 10 days as compared with 73.5 percent in water-sealed containers and 24.5 percent in loosely covered petri dishes. The daily temperature range was from 15° to 30° C.

The relation between respiratory enzyme activity and germination and viability of seeds of various plants has been extensively studied but with varying results. Crocker and Harrington (16) found that catalase and respiratory activities of Johnson and Sudan grass seeds increased as germination progressed. Furthermore, there was a close correlation between catalase activity and respiratory intensity, but not a very significant one between either of these processes and the vitality of the seeds.

The effect of fertilizers on the emergence of seedlings has been studied by Birks (5) and Maxton (34). The former found that if canary grass seed were planted in drills with superphosphate, injury to emergence resulted when the soil was moderately moist, but when the soil was either very moist or air-dry, no injury resulted. Maxton found that mixing of grass seeds with dry fertilizers did not injure the seeds to any appreciable extent and that injury to germination resulted only when the mixture was placed in moist soil.

Scarification of seed has been a universal method of improving the germination of various seeds whose coats prevent the absorption of water by the seed, hinder gaseous exchange in respiration, or prevent the maximum expansion of the embryo and its subsequent emergence from the seed proper. Many methods have been used to effect a break of the outer covering, including the use of acids and bases, mechanical scarification, removal of part of seed coat, removal of hulls of certain seeds, etc. Use of bases in either concentrated or dilute solutions has not been very successful; of the acids used, H_2SO_4 has been the most successful in scarifying seeds with intact hulls and hard coats. Using concentrated H_2SO_4 (10- to 20-minute treatment) on Bermuda grass seed, Bryan (7) improved the germination of this grass from 22.5 percent to 71 percent. With this same species, Morinaga (36) found that scarifying from 3 to 9 minutes with concentrated H_2SO_4 and germinating at a constant temperature of 27° C. obviated the necessity for light and alternating temperatures as aids in good germination. The germination of the seed of *Oryzopsis hymenoides* (Indian ricegrass) was increased by Stoddart and Wilkinson (49) by treating the seed with concentrated H_2SO_4 and by removing the seed coat. According to Toole (59), the use of the concentrated acid was detrimental but use of 71 percent acid was beneficial. Burton (8, 9) was successful in increasing the germination of Bahia grass with acid scarification using concentrations of 94 and 78 percent H_2SO_4. He obtained a similar result when the lemma and palea were removed from the seed. Ray and Stewart (43) obtained marked improvement in germination of the seed of *Paspalum dilatatum, P. floridanum,* and *P. pubiflorum* following removal of the lemma and palea.

The use of alternating temperatures to increase the germination of seeds of grasses and garden crops was extensively studied by Harrington (27). He found that alternating temperatures were unnecessary for germination of the seeds of timothy, awnless brome grass, perennial and Italian ryegrasses, and meadow fescue. He obtained best germination results for redtop, orchard grass, and Kentucky bluegrass seeds by using temperatures of 20° C. for 16 to 18 hours and 30° C. for 6 to 8 hours daily. For germination of Bermuda

grass seed, daily alternation of 20° C. and 35° C. (16 to 18 hours in the low and 6 to 8 hours in the high temperature) was best. For Johnson grass seed, a daily alternation of 30° C. for 18 to 22 hours and 45° C. for 2 to 6 hours was considered optimum. Harrington states that the beneficial effect of alternating temperatures on germination is due not to the specific effect of the extreme temperatures of the alternation or to the mean temperature of the alternation but to the changes in the temperatures. Morinaga (36) used more extreme temperatures in the alternation and found that a daily alternation of 10° to 38° C. (6 hours in the high and 18 hours in the low temperature or *vice versa*) in the dark was very beneficial to the germination of the seed of Bermuda grass. Daily alternation between temperatures of 15° to 32° C. was optimum for germination of seed of *Poa compressa* (Canada bluegrass). According to Pladeck (41), burs of buffalo grass germinated best in sterilized soil in petri dishes at daily alternation of 20° to 30° C., or 20° to 35° C. with light for 6 to 8 weeks. Trumble (62) gives optimum temperatures of germination for the seeds of many species of grass and leguminous plants in Australia. Germination of Sudan grass seed was low at a temperature range of 10° to 13° C., but very high at a range of 14° to 30° C. (47). According to Toole (59), daily alternation between 20° and 30° C. is as effective in producing germination of the seed of Indian ricegrass as scarification with 71 percent H_2SO_4. For the germination of freshly harvested seeds of several species of *Poa* and of *Dactylis glomerata,* it was found (48) that a daily alternation of 10° to 30° C. or 15° to 30° C. gave best results. Toole determined the beneficial effect of alternating temperatures upon the germination of the seeds of poverty grass (58), vine-mesquite, and plains bristle-grass (60). As shown by Toole and Toole, carpet grass seed (56) and goosegrass seed (57) also respond to alternating temperatures. They used 35° C. for carpet grass and 40° C. for goosegrass as the higher temperatures in the alternation.

The use of chemicals to break dormancy and to induce the germination of seeds has been extensive. With grass seeds, these chemicals have been confined mainly to nitrogen-carrying compounds such as KNO_3, $NaNO_3$, $NaNO_2$, and HNO_3 in dilute concentrations. Thus Morinaga (36) observed that 0.01N KNO_3 in combination with light and alternating temperatures was highly beneficial to the germination of the seeds of Bermuda grass and Canada bluegrass. Toole (58, 60, 61) and Toole and Toole (56, 57) found that 0.2 percent KNO_3 stimulated germination of grass seeds when used as a germination medium. Canada bluegrass seed germinated 60 to 70 percent in a dilute solution of HNO_3 in the dark, while the control in the dark germinated only 20 to 30 percent (2).

Selection of Species for Investigation

From 1938 to 1943, seeds of grass species, most of them believed to germinate poorly under ordinary conditions, were collected from the experimental plots of the Agronomy Division of the Hawaii Agricultural Experiment Station on Oahu and on the other islands of the Territory.[2] Seeds of a few spe-

[2] The author is indebted to the various members of the Agronomy Division for their assistance in collecting and making available the seeds investigated.

cies which do not seed very readily in Hawaii were obtained from commercial firms on the mainland United States, Australia, and elsewhere. These freshly harvested seeds were germinated in soil flats in a greenhouse at the University of Hawaii without special treatments to determine their germinative capacity under conditions quite similar to field conditions. After several trials for each species, the seeds were carefully examined, and, since very often what appears to be a normal seed is an empty floret, the "percent normal seed"[3] for each species was determined. Thereafter, every lot of seed tested was examined for percent normal seed, and the germination percentage was based on the normal seed percentage.

Fifty-two species were investigated for germination under ordinary conditions. Because of their low germination (below 60 percent) in spite of high percent normal seed (over 30), 10 were selected for further investigation as to types and methods of breaking dormancy. The remaining species were not investigated further, either because of their low percent normal seed (below 30), high germination (over 60 percent of seeds of over 30 percent normal seed), or insufficient seed supply. This bulletin deals especially with the germination studies conducted on the 10 selected species which were as follows: *Cenchrus biflorus* Roxb., *Cynodon dactylon* (L.) Pers. (Bermuda grass), *Panicum prolutum* F.v.M. (Coolah grass), *Paspalum notatum* Flugge (Bahia grass), *Pennisetum ciliare* (L.) Link, *P. setosum* (Swartz) L. Rich. (Feathery pennisetum), *Poa pratensis* L. (Kentucky bluegrass), *Sporobolus airoides* (Torr.) Torr. (Alkali sacaton), *S. wrightii* Munro (Sacaton), and *Urochloa pullulans* Stapf.

[3] As used in this bulletin, "percent normal seed" means the percentage of normal caryopses or grains as determined by number in a given lot of seeds. Since the percent normal seed is influenced by environmental conditions acting on pollination and subsequent seed development, by the degree of maturity of the seed at harvest, by the extent of insect and bird damage in the field, and by threshing and cleaning operations in the laboratory, it does not necessarily follow that the percentage found for a given sample is characteristic of the species.

GENERAL EXPERIMENTAL PROCEDURE

Since experimental procedure differed with each of the 10 species, it will be described in detail for each species. The general procedure consisted of germinating the treated seeds in soil flats in the greenhouse and in petri dishes. Each soil flat (12½ by 19 by 3½ inches) was divided into six equal compartments with removable partitions. These flats were placed in the greenhouse, and each series of experiments was planned and carried out in a randomized layout somewhat similar to that of a field experiment. Each treatment was replicated 6 to 12 times using 50 to 100 or more seeds for each replicate. Presoaking in water and in solutions was effected in test tubes at room temperature. When concentrated H_2SO_4 (C. P. 96 percent, 1.84 specific gravity) was used, the seeds after treatment were washed thoroughly in running tap water before planting or drying. When drying was required, after either the soaking or the acid treatment, it was effected at room temperature. Although various soil lots were used, they were in general similar to each other in physical and presumably in chemical make-up. Before use in germination studies, soils were steam sterilized[4] at a pressure of approximately 15 pounds per square inch for 4 hours to discourage action of damping-off fungi. In some cases soils that had been used previously and did not contain damping-off fungi were used. In all cases, however, the same soil was used for a given series of experiments. In planting, only enough soil was placed over the seeds to cover them completely (38, 32), and the flats were watered as required. The germination period was 28 days, and germination counts were made once a week. Daily soil temperature readings were taken with particular attention to maximum and minimum temperatures. Data obtained from soil experiments were subjected to variance analyses, and the degree of difference between treatment means necessary for significance at a probability of 5 percent was determined.

Attempts were made to duplicate the soil results in petri dishes using moistened filter paper as the substratum. Other tests were made to determine the cause of dormancy. These trials were necessarily on a small scale because of the tedious and delicate work involved in preparing the small seeds for the various tests. Nevertheless, tests were repeated for each species until convincing evidence was obtained. In most cases, the petri dish series were not exact counterparts of the soil series in that they were not run at the same time nor were the same lots of seed always used. This was rather unfortunate, but, because similar results were obtained in the soil and in the petri dish series with different lots of seed under the same treatment at different times, the evidence of the effect of certain treatments on germination was convincing. Germination was carried out at room temperature, in a dark Minnesota seed germinator set higher than room temperature, or at daily alternating temperatures (between room and germinator temperatures). The use of the alternating temperatures was intended more or less to duplicate the temperature

[4] The author is indebted to the Pineapple Research Institute of Hawaii for sterilizing the soils.

variations in the soil flats where the maximum temperature was higher than the maximum room temperature. Thus the temperature in the germinator was maintained at 33° C. (± 1°), this being fairly close to the average maximum in the soil. In the daily temperature alternation, the seeds were subjected to the high temperature in the germinator from 10 a.m. to 4 p.m. (6 hours) and then subjected to the low temperature (room temperature) from 4 p.m. to 10 a.m. the following day (18 hours). Because the maximum temperature of the day came before 4 p.m. and because until this hour the seeds were still in the germinator, the maximum temperature of the so-called room temperature in the alternation was slightly lower than that of the actual room temperature. This difference does not seem significant; however, for each series of experiments with temperature, the average actual daily room temperature range and also the average daily room temperature range of the alternation during the experimental period are reported. Germination periods ranged from a few days to 1, 2, 3, 4, or more weeks, and germination counts were made at intervals.

In soil and petri dish series solutions of chemicals used were made up with tap water.

The following criteria of germination were used: In petri dishes, unless otherwise stated, a seed with its primary shoot and root (both plumule and radicle) protruding through the seed coat was considered germinated, but very weak seedlings were not counted; in soil, all seeds producing shoots above the surface of the soil were considered germinated.

EXPERIMENTAL RESULTS

Sporobolus wrightii

Germination in soil

The seeds of *Sporobolus wrightii* used in germination trials in soil and in petri dishes were from a lot harvested locally on August 20, 1940. The percent normal seed was 100.

In a preliminary trial in soil after soaking in various solutions of ammonium thiocyanate, sucrose, "Vita-flor" (commercial product containing vitamin B complex), potassium nitrate, vitamin C, vitamin B_1, indole-3-acetic acid, and ethyl alcohol in various concentrations, only "Vita-flor" and sucrose seemed to increase germination, and this but slightly. In a large replicated test, however, soaking seeds in various concentrations of these solutions for 24 hours at room temperature did not improve germination above that of the seeds soaked in tap water. Tap-water soaking increased germination from about 35 percent in the dry control to about 62 percent in the soaked.

The optimum length of soaking period for maximum germination was determined. Seeds were soaked for 24, 48, and 72 hours in tap water, and before planting, one lot of seed was dried to determine the effect of drying on germination. The results are recorded in table 1.

TABLE 1. Effect of soaking in tap water and subsequent drying on germination of seed of *Sporobolus wrightii.*

Replications: 12 (100 seeds each).
Germination in freshly sterilized soil.
Germination period: January 15, 1941, to February 12, 1941.
Average daily soil temperature range: 18.6° to 30.2° C.

TREATMENT	AVERAGE GERMINATION[1]
	Percent
Tap water 24 hours	68.8
Tap water 48 hours	67.5
Tap water 72 hours	68.9
Tap water 24 hours, dry 48 hours	60.2
Dry control	55.2

[1] Difference of 6.4 percent necessary for significance.

According to table 1, 24-hour soaking was as effective as the 48-hour and 72-hour soaking in increasing germination of the seed of this species. The table also shows that drying the seed after soaking largely offsets the advantages derived from soaking. This is in opposition to the results of Wenger (63) who found that drying the seed of buffalo grass after soaking in tap water materially increased germination.

To determine whether scarification of the seed would produce the same result as soaking in tap water, seeds were soaked in concentrated sulphuric acid (H_2SO_4) for two minutes before planting. The results showed that acid scarification was ineffective but that soaking in water was effective in increasing germination.

Germination in petri dishes

Lots of seed were treated with concentrated H_2SO_4 for periods ranging from 1 to 10 minutes and germinated on moistened filter paper in petri dishes at room temperature. After one week, the optimum treatment (4-minute treatment) resulted in a germination of about 21 percent as compared with about 2 percent germination of the control. About 32 percent of the seeds in this treatment was injured by the acid, however, while all of the ungerminated seeds in the control were sound. Suspecting that the low germination in the control as compared with that in the soil was due to the lower temperature in the petri dishes, the series was then alternated between room and germinator temperatures. In one week, the optimum acid treatment (1-minute treatment) produced a total germination of 69 percent and the control 74 percent. Thus, two facts which are in agreement with the germination results in soil were brought to light: First, acid scarification is of no benefit in improving germination, and second, temperature is the determining factor in germination of seed of this species. The average daily maximum room temperature of approximately 24° C. was too low for germination, but when this maximum was raised in the germinator to 33° C. for six hours of the day, the resulting germination was high. The better germination in the petri dishes may have been due to the higher temperature to which the seeds were subjected.

In an attempt to determine the effect of the lemma and palea on the absorption of water by the seed, intact seeds and seeds with the lemma and palea removed were soaked in tap water at room temperature for 48 hours and the amount of water absorbed by the seeds determined. The lemma and palea did not interfere with the water intake by the caryopsis which absorbed water to approximately 2½ times its air-dry weight. The lemma and palea became soaked and could be detached easily from the caryopsis. The pericarp became gelatinous.

After the permeability of the seed coat to water was determined, the seeds that were soaked with the lemma and palea intact were germinated at room temperature, constant germinator temperature, and at alternating temperatures. The results are recorded in table 2.

TABLE 2. Effect of temperature on the germination of the seed of *Sporobolus wrightii* pre-soaked in tap water for 48 hours at room temperature.

Replications: 4 (100 seeds each).
Germination in petri dishes.
Germination period: February 14, 1941, to February 21, 1941.

TREATMENT	AVERAGE GERMINATION AT THE END OF —		
	2nd day	4th day	7th day
	Percent	*Percent*	*Percent*
Room temperature: (21.5°—27.5° C.)	0.0	6.5	8.8
Constant germinator temperature: (33° C.)	34.2	38.4	40.2
Alternating temperature: (21.5°—26.5° C. to 33° C.)	19.5	70.7	81.0

The effect of alternating temperatures on germination of the seed of *Sporobolus wrightii* is very pronounced (table 2).

When the seed is germinated under the less favorable temperature conditions found in the soil, the stimulation derived from soaking becomes apparent.

In an attempt to determine the cause of low germination at room temperature, some seeds were germinated at room temperature for one week. The resulting germination was 12 percent. Then a small cut was made with a sharp knife in the seed coats of the ungerminated seeds on the end opposite the germ and the germination was continued for another week. This treatment brought about the germination of an additional 36 percent. Then the seeds were given the alternating temperature treatment resulting in an additional 29 percent germination in a week, bringing the total germination to 77 percent. Thus it seems that the cause of dormancy of the seed lies in the character of the seed coat. Since the seed absorbed a tremendous amount of water as was shown previously, the reduction in germination was not due to inadequate absorption of water. Furthermore, the seed was found to be fully swollen when the incision in the seed coat was made, yet, low germination at room temperature resulted. The increase in germination brought about by the incision in the seed coat was probably due to the increased facility for gas exchange involved in the process of respiration as was found in sulphuric-acid-treated seed of poverty grass (58). Toole (61) makes a similar statement. The role of alternating temperatures may be of similar nature, since at the constant higher temperature of 33° C. (table 2), germination was much better than at room temperature. Crocker (12) has shown that high temperature (33° C.) increased the germination of intact seed of *Xanthium* (cocklebur) by increasing the rate of oxygen absorption through the seed coat and by raising the respiratory quotient, the former in spite of the fact that the solubility of any gas in water decreases with the increase in temperature. Later, Shull (46) also working with cocklebur seed, found that the minimum oxygen requirement for germination decreased with the increase in temperature. The exact role that alternating temperatures play in seed germination has not yet been explained fully.

Summary

Germination of the seed of *Sporobolus wrightii* is hindered by inadequate gas exchange (oxygen intake and carbon dioxide output) through the seed coat. When the seed coat is modified by soaking in water for 24 hours, by cutting, or by subjecting the seed to daily alternating temperatures (18 hours at room temperature and 6 hours at 33° C.), the necessary gas exchange involved in the process of respiration is facilitated and germination is increased.

For field planting, the soaking-in-water treatment seems to be most practical. The other methods may be used by the seed analyst to obtain the potential germination percentage of this species.

Sporobolus airoides

Germination in soil

The seeds of this species used in these germination experiments (soil and petri dish series) were of one lot harvested locally on August 9, 1940. Percent normal seed was 100.

In a preliminary trial in which various treatments were employed, it was observed that soaking the seed in dilute solutions of potassium nitrate (KNO_3),

vitamin C, and ethyl alcohol before planting seemed to stimulate germination. This trial was followed by a large replicated test in which various concentrations of the solutions were used. The treatments and the germination results are recorded in table 3.

TABLE 3. Effect of pre-soaking (24 hours) in various solutions on germination of seed of *Sporobolus airoides*.

Replications: 12 (100 seeds each).
Germination in used sterilized soil.
Germination period: December 7, 1940, to January 4, 1941.
Average daily soil temperature range: 20.5° to 28.7° C.

TREATMENT	AVERAGE GERMINATION[1]
	Percent
0.5 percent KNO_3	53.8
1 percent KNO_3	58.2
2 percent KNO_3	59.0
0.25 percent vitamin C	41.6
0.5 percent vitamin C	45.3
1 percent vitamin C	45.6
2.5 percent ethyl alcohol	33.8
5 percent ethyl alcohol	29.7
10 percent ethyl alcohol	24.9
Tap water	30.8
Dry control	23.1

[1] Difference of 7.4 percent necessary for significance.

Table 3 shows that KNO_3 and vitamin C produced significantly higher germination than tap water. The three concentrations of the chemicals varied little in effect.

Since KNO_3 was superior to vitamin C, it was used in 1 percent solution in an experiment in which the time of pre-soaking was varied. The effect of drying after the soaking treatment was determined, since from the practical standpoint, dry seed can be sown much more easily than wet seed. The results showed that whether the seed was soaked in KNO_3 for 24, 48, or 72 hours, the effect was about the same. The 3-day soaking is not to be recommended, since some seeds germinated during the soaking period. Seed soaked in KNO_3 and dried for 48 hours before planting gave about the same germination figure as that of the undried seed. On the other hand, seed soaked in tap water and dried before planting showed a reduction in germination which approximated that of the dry control.

Because KNO_3 was effective even after drying the treated seed, it seemed desirable to determine the effect of various periods of drying upon germination. In addition, the effect of sulphuric acid (H_2SO_4) scarification was determined. Table 4 gives the treatments and results.

From table 4, it is clearly seen that the nitrate is effective even after drying as compared with the water-soak control and the dry control; drying for 1 to 3 days had no effect on the ability of the nitrate treatment to promote germination. The germination of the nitrate-treated seed 13 days after being stored dry at room temperature was a little above 70 percent. Thus there is the possibility of keeping the treated seed for some time before planting without loss in effectiveness. Table 4 shows that the acid treatment was generally ineffective, and although soaking the acid-treated seed in tap water produced a significantly higher germination than that of the dry control, this figure was no higher than that for the nitrate-treated seed.

TABLE 4. Effect of drying after soaking in KNO_3 solution and of acid scarification on germination of *Sporobolus airoides* seed.

Replications: 6 (100 seeds each).
Germination in used sterilized soil.
Germination period: February 19, 1941, to March 19, 1941.
Average daily soil temperature range: 19.6° to 31.6° C.

TREATMENT	AVERAGE GERMINATION[1]
	Percent
1 percent KNO_3 24 hours, dry 24 hours	58.5
1 percent KNO_3 24 hours, dry 48 hours	67.7
1 percent KNO_3 24 hours, dry 72 hours	65.3
Tap water 24 hours, dry 24 hours	49.2
Tap water 24 hours, dry 48 hours	50.2
Tap water 24 hours, dry 72 hours	42.7
Concentrated H_2SO_4 1 minute	52.5
Concentrated H_2SO_4 1 minute, dry 24 hours	51.3
Concentrated H_2SO_4 1 minute, dry 48 hours	46.5
Concentrated H_2SO_4 1 minute, 1 percent KNO_3 24 hours	54.3
Concentrated H_2SO_4 1 minute, 1 percent KNO_3 24 hours, dry 24 hours	30.3
Concentrated H_2SO_4 1 minute, tap water 24 hours	64.2
Concentrated H_2SO_4 1 minute, tap water 24 hours, dry 24 hours	48.5
Dry control	45.2

[1] Difference of 10.6 percent necessary for significance.

Germination in petri dishes

Seeds were treated with concentrated H_2SO_4 for varying periods from 1 to 20 minutes and germinated in petri dishes at room temperature. In one week, the one-minute treatment produced the highest germination of slightly more than 60 percent as compared to the control germination of only 8 percent. This series was repeated with similar results. It will be recalled that the acid-treated seed planted in soil did not show increase in germination over that of the untreated seed (table 4). Furthermore, the germination of the acid-treated seed in the petri dishes was nearly equal to that of the nitrate-treated seed in the soil. Also the germination of the untreated seed in the petri dishes (8 percent) was very much lower than that of the untreated in the soil (45 percent).

It was suspected that increased permeability of the seed coat to water absorption may be responsible for the increased germination with the acid treatment in the petri dishes. It was soon discovered that although the acid destroyed part of the thin, loose lemma and palea and seed coat, the acid-treated seed absorbed no more water than the control, which upon soaking, became fully swollen.

It has been suggested by Cashmore (10) that KNO_3 may help to increase the permeability of the seed coat and thus promote germination. Although this possibility seemed very doubtful, it was given a trial. With the seed of *Sporobolus airoides* and other seeds that responded to the nitrate treatment in the present studies, it may be said that within a given period, none of the nitrate-treated seeds absorbed more water than the untreated seeds soaked in pure water.

In one of the trials made to determine the absorption of water by the nitrate-soaked seed and the water-soaked seed, the seeds were germinated in water in petri dishes at room temperature after the absorption trial. Sixteen percent of the seeds soaked in 1 percent KNO_3 germinated while only 5 percent of the

seeds soaked in tap water germinated in two weeks. When the seed coats of the ungerminated seeds of the two treatments were cut, 53 percent of the nitrate-soaked seed germinated while 70 percent of the water-soaked seed germinated in one week, bringing the total of the KNO_3 treated to 69 percent and that of the water-soaked to 75 percent. Thus it seemed that the nitrate was effective, but to a lesser degree than in the soil, and that cutting the seed coat had about the same effect as the acid treatment in the petri dishes.

The rather poor response shown by the nitrate-treated seed and by the untreated seed in the petri dishes as compared with the response in the soil was then suspected to be due to differences in the temperature of the germinating medium. With this point in view, a series roughly comparable to the soil series was conducted. The nitrate-soaked seeds and the water-soaked seeds were germinated at three temperature conditions: Continuous room temperature, continuous germinator temperature, and alternating between the two. The results obtained in one week are tabulated in table 5.

TABLE 5. Effect of temperature on the germination of the seed of *Sporobolus airoides* as affected by pre-soaking in KNO_3 and tap water.

Replications: 4 (100 seeds each).
Germination in petri dishes.
Germination period: February 12, 1941, to February 19, 1941.

TREATMENT	AVERAGE GERMINATION IN —	
	3 days	7 days
	Percent	*Percent*
Continuous room temperature (21.4°—26.9° C.):		
1 percent KNO_3 24 hours	7.0	14.2
1 percent KNO_3 24 hours, dry 48 hours	2.0	7.5
Tap water 24 hours	4.0	6.0
Tap water 24 hours, dry 48 hours	0.8	3.0
Continuous germinator temperature (33° C.):		
1 percent KNO_3 24 hours	26.0	54.8
1 percent KNO_3 24 hours, dry 48 hours	17.2	41.0
Tap water 24 hours	18.5	31.5
Tap water 24 hours, dry 48 hours	19.2	35.0
Alternating temperature (21.4°—26.0° C. and 33° C.):		
1 percent KNO_3 24 hours	43.8	72.2
1 percent KNO_3 24 hours, dry 48 hours	39.0	66.5
Tap water 24 hours	27.2	52.2
Tap water 24 hours, dry 48 hours	25.0	58.0

It is seen from table 5 that temperature has a marked influence on the germination of the seeds of *Sporobolus airoides*. The ineffectiveness of KNO_3 at continuous room temperature is demonstrated again. At the constant high temperature and at alternating temperatures, the nitrate was very effective in promoting germination. Better germination occurred under the alternation than at the constant high temperature. Water-soaked seed also germinated better under the alternation than at either the constant room temperature or the constant high temperature. Drying the seed had no effect on the effectiveness of the nitrate treatment under the alternating temperature condition.

If the germination results obtained with KNO_3 in soil (table 4) are compared with those obtained with the same treatment in petri dishes under tem-

perature alternation (table 5), it will be seen that these results are quite similar. Germination obtained with the water-soaked seed in soil and in petri dishes is also similar. This consistency seems to have resulted from the similarity of the temperature conditions in the two series—in the soil the range was 20° to 32° C., and in the petri dishes it was 21° to 33° C. The poorer germination shown in table 3 was probably the result of the lower maximum temperature of the soil.

In another petri dish series, it was shown that KNO_3 was effective even after washing the soaked seeds thoroughly in running water, indicating that the salt was absorbed by the seed or that the stimulus to germination was initiated while the seeds were being soaked in the solution. The former postulate seems the more likely, since it has been shown that dried nitrate-treated seeds germinated better than dried, water-soaked seeds even after about two weeks of storage. A combination of the two theories is not improbable, however.

Recapitulating, it seems that dormancy in the seed of *Sporobolus airoides* is due to some inherent condition of the seed coat which at room temperature hinders the exchange of gases involved in the process of respiration. When the seed coat is cut, the seed germinates readily even at room temperature. Toole (61) states that in this and other species of *Sporobolus,* the seed coats may hinder gaseous exchange. Dormancy is also partly broken at high temperature as was the case in cocklebur seed (12) which increased the intake of oxygen with an increase in temperature. The further beneficial role of alternating temperatures in inducing germination has not been determined, but it is likely that the gaseous exchange through the seed coat is involved. Finally the greatest amount of stimulation is obtained by a combined treatment of KNO_3 and alternating temperatures. The direct function of KNO_3 in the respiratory process is not clear, but this salt may serve as a ready source of nitrogen or oxygen which combines with the breakdown products of the carbohydrate molecule to form amino acids in the synthesis of proteins and subsequent growth (germination) of the embryo.

Summary

The seed coat of *Sporobolus airoides* obstructs exchange of gases and delays germination. Modification of the seed coat by soaking in water for 24 hours, by scarifying with concentrated H_2SO_4 for one minute, or by cutting, improves germination. Subjecting the seed to alternation between daily room temperature and 33° C. also improves germination. Soaking for 24 hours in 1 percent KNO_3 solution which may act in a nutritive capacity further increases germination when used in combination with alternating temperatures. Soaking for 24 hours in 0.5 percent or 1 percent vitamin C solution (ascorbic acid) also helps the germination of this species. The nitrate treatment is effective even after washing or drying the seed following soaking in the solution.

The potassium nitrate treatment is the best for practical application. To facilitate sowing, the seed should be dried after soaking in the chemical. This and the other treatments may be used in the laboratory to determine maximum germination of this seed.

Poa pratensis

Germination in soil

Harrington (27) using *Poa pratensis* and Sprague (48) using other species of *Poa* found that alternating temperatures were beneficial in inducing the

germination of the seeds of these species. Morinaga (36) combined temperature alternation with KNO_3 and light to obtain good germination with the seed of *Poa compressa* on moist filter paper. In the present instance it was desirable to test the effect of these treatments on germination in soil.

The seeds used in the soil series and in the petri dish series were part of a lot obtained from a mainland United States seed firm on July 5, 1940, and presumably were fresh. The percent normal seed was 48.

In two preliminary trials in which KNO_3 was used in addition to other treatments (ammonium thiocyanate, sucrose, vitamin B_1, heteroauxin, ethyl alcohol, amino acids, and other nitrogen carriers), only KNO_3 seemed effective in increasing the germination of *Poa pratensis* in soil. In a replicated series using various concentrations of this salt (0.5, 1, and 2 percent solutions) and various soaking periods (24, 48, and 72 hours), it was found that treating the seed in KNO_3 solution regardless of the strength used and length of soaking period, did not increase its germination above that of the seed soaked in water for the same periods. However, the water-soaked seed as well as the nitrate-treated seed germinated much better than the dry control. Water-soaked seed germinated better than 60 percent, while the dry control germinated approximately 41 percent. When the nitrate-treated seed was dried for two days before planting, its germination was still about the same as that of the undried seed, but when the water-soaked seed was dried for a similar period before planting, its germination dropped approximately to that of the dry control. The soil temperature ranged from 20.1° to 29.9° C.

After the determination of the effectiveness of dried nitrate-treated seed, another replicated series was conducted soaking the seed in a 2 percent solution of the salt for 48 hours and then dried for 24, 48, and 72 hours before planting. A counterpart in which tap water was used was maintained as a control in addition to the dry control. This time the nitrate treatment was not as effective as in the previous experiment and germination was no better than in the dry control. The germination of the water-soaked and dried seed was, on the other hand, much superior to that of the nitrate-treated and dry control seeds. Length of drying period made no difference in the effectiveness of the water-soaked seed, which germinated more than 50 percent, comparing favorably with the 35 percent germination of the dry control. The average soil temperature range during this experiment was 19.0° to 31.6° C. as compared with 20.1° to 29.9° C. range of the previous experiment. This difference in temperature range seems to have caused the divergent results obtained in the two experiments.

Germination in petri dishes

As with *Sporobolus airoides*, the seed of *Poa pratensis* did not absorb any more water when soaked in a 2 percent solution of KNO_3 for 48 hours than when soaked in tap water. In a permeability trial over a 48-hour period, seed soaked in the nitrate solution absorbed water to approximately 65 percent of its air-dry weight, and the water-soaked seed absorbed approximately 68 percent of its air-dry weight. When the nitrate-soaked and water-soaked seeds were germinated in petri dishes at room temperature with tap water as substratum, the former germinated 51 percent and the latter 40 percent.

In another experiment the nitrate-treated seeds were germinated at room temperature, germinator temperature, and alternating temperature. The germination results are recorded in table 6.

TABLE 6. Effect of temperature variables on germination of *Poa pratensis* seed pre-soaked in 2 percent KNO_3 for 48 hours.

Replications: 4 (100 seeds each).
Germination in petri dishes.
Germination period: February 20, 1941, to March 6, 1941.

TREATMENT	AVERAGE GERMINATION IN —	
	1 week	2 weeks
	Percent	*Percent*
Continuous room temperature (21.9°—26.4° C.):		
2 percent KNO_3 48 hours	24.5	54.7
2 percent KNO_3 48 hours, dry 48 hours	26.6	66.2
Tap water 48 hours	15.6	28.6
Tap water 48 hours, dry 48 hours	20.3	46.4
Continuous germinator temperature (33° C.):		
2 percent KNO_3 48 hours	1.0	1.0
2 percent KNO_3 48 hours, dry 48 hours	0.5	0.5
Tap water 48 hours	0.5	0.5
Tap water 48 hours, dry 48 hours	0.0	0.0
Alternating temperature (21.9°—26.2° C. and 33° C.):		
2 percent KNO_3 48 hours	30.2	45.3
2 percent KNO_3 48 hours, dry 48 hours	23.4	60.4
Tap water 48 hours	20.3	47.9
Tap water 48 hours, dry 48 hours	17.7	40.1

In table 6, it is seen that in general alternating temperatures were no better than room temperature in germinating the seed of *Poa pratensis* and continuous exposure of the seed to the higher temperature of the alternation was definitely detrimental. How much of this detrimental effect was due to the darkness in the germinator is yet to be determined. It will be remembered that with the seed of *Poa compressa,* Morinaga (36) found light to be advantageous to germination. A further examination of the data presented in table 6 shows that drying of the nitrate-treated seed before germinating resulted in an increase in germination over that of the undried seed.

Although direct comparisons between the results obtained in the soil and those obtained in the petri dishes cannot be made because of the differences in the temperature ranges occurring during the experimental periods, it can be said that germination of the seed of *Poa pratensis* is affected by temperature and KNO_3 in soil as well as in a petri dish.

Washing the nitrate-soaked seed in running tap water did not reduce effect of the treatment, since germination was 37 percent as compared with 20 percent for the seed soaked in water and washed and germinated at room temperature.

The possible role of KNO_3 and alternating temperatures in affecting germination of the seed of *Sporobolus airoides* applies equally well to the seed of *Poa pratensis.*

Summary

Delayed germination of the seed of *Poa pratensis* is probably due to the nature of the seed coat which prevents the free gas exchange of the seed and

the need, perhaps, of some food source. The seed coat may be modified by soaking the seed in water for 24 hours or by subjecting the seed to daily alternating temperatures. Food source is made available by treating the seed in 2 percent KNO_3 solution for 48 hours, good germination occurring even at room temperature. Washing or drying the seed after the nitrate treatment does not alter the effectiveness of the treatment.

For testing the seed of this grass in the laboratory, all of these methods may be employed, but for field application, the potassium nitrate treatment in which the soaked seed is dried before sowing is recommended.

Cynodon dactylon

Germination in soil

Although the germination behavior of the seed of *Cynodon dactylon* has been worked out by Harrington (*27*) and Morinaga (*36*) under laboratory conditions, it is of interest to determine the behavior of the seed in soil.

The seed used in the following soil and petri dish experiments was of the same lot received from a mainland United States seed firm on July 5, 1940, and presumably fresh when procured. Seed was "unhulled," that is, with the lemma and palea intact on the caryopsis. Percent normal seed was 100.

In a preliminary trial using the same treatments as in the preliminary trials of *Poa pratensis* seed, it was found that only KNO_3 increased germination of *Cynodon dactylon* seed. In a replicated series that followed, 1 and 2 percent KNO_3 treatment (24 hours) resulted in 81 percent germination, while tap water soaking and dry control produced 70 and 51 percent, respectively. The soil temperature range during the experiment was 23.6° to 32.4° C.

The effect of prolonged soaking and of drying after the nitrate treatment was studied, since if the treatment proved effective even after drying, it would be of value in practical application to field planting. Table 7 gives the results of the experiment.

TABLE 7. Germination of *Cynodon dactylon* seed as affected by drying after soaking in KNO_3 solution.

Replications: 12 (50 seeds each).
Germination in used sterilized soil.
Germination period: October 17, 1940, to November 14, 1940.
Average daily soil temperature range: 23.1° to 33.0° C.

TREATMENT	AVERAGE GERMINATION[1]
	Percent
1 percent KNO_3 24 hours	83.2
1 percent KNO_3 48 hours	83.5
1 percent KNO_3 72 hours	85.2
1 percent KNO_3 24 hours, dry 24 hours	77.0
1 percent KNO_3 48 hours, dry 48 hours	81.3
1 percent KNO_3 72 hours, dry 72 hours	80.7
Tap water 24 hours	82.7
Tap water 48 hours	80.3
Tap water 72 hours	79.5
Tap water 24 hours, dry 24 hours	78.3
Tap water 48 hours, dry 48 hours	77.8
Tap water 72 hours, dry 72 hours	65.8
Dry control	59.7

[1] Difference of 5.2 percent necessary for significance.

In table 7, it is seen that under the temperature conditions prevailing during the experimental period, the nitrate-treated seed planted without drying did not, in general, germinate any better than the water-soaked controls, although the 72-hour nitrate treatment seemed slightly superior to the 72-hour water control. The point of most significance in this experiment is that regardless of the length of the drying period up to three days, the effect of the nitrate was still manifested. The water-soaked seed, with increase of the drying period, showed a gradual decrease in germination percentage approaching the figure for the dry control.

In another series, the seeds were dried for 24, 48, 72, and 96 hours after the nitrate soaking. Behavior of the dried nitrate- and water-soaked seeds shown in the preceding experiment was verified in this experiment. Although in the preceding experiment, the 3-day drying of the water-soaked seed approached the dry control in germination, in this experiment an additional day of drying resulted in the germination of the dried seed about equal to that of the dry control. Length of the drying period of the nitrate-treated seed still had no significant effect on germinative capacity.

Nitrate-treated seed germinated 81 percent after dry storage at room temperature for 65 days, and an untreated lot germinated 67 percent after the same period of storage. This suggests that drying of the nitrate-treated seed has a practical value.

Although Bryan (7) and Morinaga (36) report the use of concentrated H_2SO_4 to scarify and induce germination of *Cynodon dactylon* seed, it was found that with the seed used here, a treatment period of one minute did not affect germination but a 3-minute soaking killed nearly all of the seeds when germinated in soil.

Germination in petri dishes

As with the seeds in soil, scarification treatment with concentrated H_2SO_4 for periods ranging from 1 to 7 minutes failed to increase the germination of this species in petri dishes at room temperature. It is of interest to note that scarification with acid for periods ranging from 3 to 9 minutes (36) and from 10 to 20 minutes (7) have been found to improve the germination of this species elsewhere.

A series was conducted to determine the effect of temperature variation on the germination of nitrate-soaked and water-soaked seed. The results of the experiment are recorded in table 8.

The effect of temperature (table 8) on the germination of the seed of *Cynodon dactylon* is very pronounced. The extreme temperatures of the alternation, that is, room temperature and 33° C., inhibited germination of both nitrate-treated and water-soaked seeds. These temperatures when used in the daily alternation resulted, however, in a very appreciable increase in germination. The nitrate treatment, dried and undried, was superior to the water-soaked treatment under the daily temperature alternation, although drying of the nitrate-treated seed reduced the germination somewhat. When the ungerminated seeds from the room temperature and germinator treatments were subjected to the daily alternation, a large percentage of them germinated to an extent comparable to that of the seeds which had been under the alternating temperatures from the beginning.

In soil where the temperature fluctuation was somewhat comparable to the alternating temperatures of the petri dish series, KNO_3 was found to be effec-

TABLE 8. Effect of temperature on germination of *Cynodon dactylon* seed pre-soaked in KNO_3 solution.

Replications: 4 (100 seeds each).
Germination in petri dishes.
Germination period: February 20, 1941, to March 13, 1941; March 13, 1941, to March 27, 1941.

TREATMENT	AVERAGE GERMINATION IN —			AVERAGE GERMINATION IN 2 WEEKS AT ALTERNATING TEMPERATURE[1]
	1 week	2 weeks	3 weeks	
	Percent	*Percent*	*Percent*	*Percent*
Continuous room temperature (21.9°—26.2° C.):				
1 percent KNO_3 24 hours	0.0	0.0	0.0	69.0
1 percent KNO_3 24 hours, dry 48 hours	0.0	0.0	0.0	33.2
Tap water 24 hours	0.0	1.0	1.0	35.2
Tap water 24 hours, dry 48 hours	0.0	0.2	0.2	29.0
Continuous germinator temperature (33° C.):				
1 percent KNO_3 24 hours	3.0	3.2	3.4	45.5
1 percent KNO_3 24 hours, dry 48 hours	1.8	2.0	2.0	32.5
Tap water 24 hours	0.0	0.5	0.5	34.2
Tap water 24 hours, dry 48 hours	0.2	0.2	0.2	26.8
Alternating temperature (21.9°—25.9° C. to 33° C.):				
1 percent KNO_3 24 hours	38.8	66.8	72.8	.....
1 percent KNO_3 24 hours, dry 48 hours	36.0	54.5	58.7	.....
Tap water 24 hours	3.0	14.8	18.0	.....
Tap water 24 hours, dry 48 hours	6.2	18.0	21.0	.....

[1] 22.5°—26.4° C. and 33° C.

tive in promoting germination as was shown previously. In the petri dishes, a similar response occurred with daily temperature alternation. The maximum soil temperature and the high temperature in the daily alternation were approximately the same (33° C.). Minimum temperatures were also similar (about 23° C. for the soil and about 22° C. for the petri dish series). These temperatures can be compared with an alternation of 20° and 35° C. recommended by Harrington (27) and with an alternation of room temperature and 38° C. in combination with KNO_3 and light recommended by Morinaga (36) for the maximum germination of *Cynodon dactylon* seed.

As to the absorption of water, in one of the tests, seed soaked in 1 percent KNO_3 absorbed approximately 42 percent of its air-dry weight in 24 hours, and seed soaked in tap water imbibed approximately 44 percent of its air-dry weight in the same period.

A lot of seed which was soaked in 1 percent KNO_3 for 24 hours was thoroughly washed with running tap water and germinated under alternating temperature conditions. The resulting germination was better than in the lot soaked in tap water and washed, although the difference between the two treatments was less marked than when the seeds were soaked in the nitrate solution and in tap water and germinated without washing. Probably some of the salt was absorbed by the seed.

Summary

In the seed of *Cynodon dactylon,* retardation in germination is caused by the gas-impervious nature of the seed coat and, perhaps, by the lack of some food

source. The seed coat is made permeable to gas by soaking the seed in water for 24 hours or by subjecting the seed to daily alternating temperatures. Soaking in 1 percent KNO_3 solution for 24 hours may afford contact with a good source of nitrogen, and in combination with alternating temperatures, results in excellent germination. The nitrate treatment is effective even when washing or drying follows soaking in the solution. Germination of this species is also improved by reduced oxygen pressure, according to Morinaga (37).

Soaking in potassium nitrate solution followed by drying before planting seems to be a treatment with practical application for improving the germination of this species. This and the other treatments may be used by the seed analyst.

Pennisetum setosum

Germination in soil

Seed of *Pennisetum setosum* used for soil germination studies was harvested locally on February 15, 1940. Its percent normal seed was 100.

The possibility that the shiny waxy surface of the seed coat (caryopsis with no attached hull) prevents the absorption of water and exchange of gas was investigated by treating the seed with concentrated H_2SO_4. A 2-minute treatment with the acid did not affect normal germination, but a 5-minute treatment was definitely detrimental to germination in soil.

In a preliminary trial using dilute solutions of various chemicals (ammonium thiocyanate, potassium nitrate, sucrose, ethyl alcohol, heteroauxin, vitamin C, thiourea, sodium thiocyanate, l-asparagin, l-leucine, nicotinic acid, glycine, and other nitrogen carriers), it appeared that ammonium thiocyanate (NH_4SCN), KNO_3, sucrose, and vitamin C were beneficial to germination. A replicated series of treatments was conducted using these substances in different concentrations. The results are shown in table 9.

TABLE 9. Effect on germination of pre-soaking seed of *Pennisetum setosum* in dilute solutions of various chemicals for 24 hours.

Replications: 12 (100 seeds each).
Germination in used sterilized soil.
Germination period: May 21, 1940, to June 18, 1940.
Average daily maximum soil temperature: 34° C.

TREATMENT	AVERAGE GERMINATION[1]
	Percent
0.5 percent KNO_3	64.3
1.0 percent KNO_3	67.0
2.5 percent sucrose	57.3
5.0 percent sucrose	53.8
0.5 percent NH_4SCN	87.2
1.0 percent NH_4SCN	92.4
0.5 percent vitamin C	66.4
Tap water	44.5
Dry control	51.6

[1] Difference of 7.8 percent necessary for significance.

That NH_4SCN was the most effective chemical in promoting the germination of the seed of *Pennisetum setosum* is apparent (table 9). KNO_3 and vitamin C were also effective, but to a lesser degree. Sucrose was no better than the dry control, and soaking in tap water had no effect on germination.

Because of its marked effectiveness in promoting germination, NH_4SCN was investigated further. Various cyanides are known to affect respiration of

plant tissues, hence it was deduced that if NH_4SCN were affecting respiration of the seed, its effect would be subject to temperature variation. Two sets of experiments were conducted in conjunction with the study of the effect on germination of drying after the treatment. One series was conducted in a greenhouse where the soil flats were exposed to sunlight from shortly after sunrise to sunset. The other series was conducted outside along the greenhouse where the flats were exposed to sunlight only until mid-afternoon, after which time they were in the deep shade of the greenhouse. The germination results obtained under these conditions are tabulated in table 10.

TABLE 10. Effect of drying and temperature on germination of thiocyanate-treated seeds of *Pennisetum setosum*.

Replications: 12 (100 seeds each).
Germination in freshly sterilized soil.
Germination period: June 12, 1940, to July 10, 1940.
Average daily soil temperature range:
inside greenhouse 22.5° to 36.5° C.
outside greenhouse 21.0° to 37.9° C.

LOCATION	TREATMENT	AVERAGE GERMINATION[1]
		Percent
Inside greenhouse	1 percent NH_4SCN 24 hours	58.7
	1 percent NH_4SCN 24 hours, dry 24 hours	77.9
	Tap water 24 hours	51.8
	Tap water 24 hours, dry 24 hours	44.7
	Dry control	38.5
Outside greenhouse	1 percent NH_4SCN 24 hours	75.1
	1 percent NH_4SCN 24 hours, dry 24 hours	84.4
	Tap water 24 hours	75.1
	Tap water 24 hours, dry 24 hours	75.2
	Dry control	66.4

[1] Difference of 7.9 percent necessary for significance.

If the germination of the treatments inside the greenhouse is compared with that of the treatments outside, it is seen that the latter is, in general, greater than the former (table 10). Although the average daily minimum and maximum soil temperatures were approximately the same in the two series (22.5° to 36.5° C. inside the greenhouse and 21.0° to 37.9° C. outside), this difference in germination occurred between the two series.

A critical analysis of the daily march of soil temperatures in the two series showed that up to about three o'clock in the afternoon when the maximum temperature was reached, little difference between inside and outside temperatures was noted. From three o'clock on, however, the soil flats outside the greenhouse were in deep shade resulting in a rapid drop in temperature. The soil flats inside the greenhouse remained at high temperature (though lower than maximum) because of continuous exposure to sunlight until sunset. From 4 to 5:30 p.m., the soil temperature inside the greenhouse was 3.3° C. higher than the soil temperature outside. This higher temperature prevailed, though to a lesser degree, from after sunset to about 5:30 the following morning when the minimum temperature was registered. Thus it seems that the higher germination obtained in flats outside the greenhouse was probably due to the lowering of the soil temperature after 3 p.m. by the shade, since the

daily minimum and maximum temperatures of the two series were approximately the same.

Under both temperature conditions, seed soaked in the thiocyanate solution and planted without drying (table 10), germinated no better than water-soaked seed. If, however, the seed were dried for 24 hours after soaking, there was a marked increase in germination over the water-soaked seed. A typical response of the seedlings developing from the thiocyanate-treated seeds was that during the earlier part of the germination period in particular, they burned off at the tips and eventually died. Mortality of seedlings was greater under the longer exposure of the soil flats to the high temperature inside the greenhouse. This suggested that high soil temperature caused the death of the young seedlings as well as the reduced germination of the thiocyanate-treated seeds.

Temperature as the factor was ruled out by the result of a small series in which NH_4SCN was again used, but this time the soil flats were shaded with rough-weave cheesecloth to reduce sunlight intensity. The average temperature range was 21.8° to 29.0° C. Even under this low temperature range, the characteristic burning of the tips of the seedlings and a reduction in germination occurred. An alternative causal factor was the soil. It will be recalled that in the first replicated test (table 9) in which the thiocyanate treatment was very effective, the soil used had been used previously, whereas in the temperature variable series (table 10), germination was carried out in freshly sterilized soil (unused); furthermore, in the last shaded series reported above, freshly sterilized soil was again employed. Only in the last two cases did the detrimental effect of the thiocyanate treatment occur.

Because Johnson (28) and Kelley and McGeorge (30), the latter working with Hawaiian soils, found that the ammonia content of soils increased with heating, an experiment was designed to test the possibility that ammonia was the toxic agent affecting germination in freshly sterilized soil. The soil flats were shaded with cheesecloth to reduce the high sunlight intensity and to keep the soil temperature below the normal level. The germination results are recorded in table 11.

TABLE 11. Effect of freshly steam-sterilized soil upon the germination of thiocyanate-treated seed of *Pennisetum setosum* as influenced by washing and drying preparatory to planting.

Replications: 12 (50 seeds each).
Germination in freshly sterilized soil.
Germination period: July 5, 1940, to August 2, 1940.
Average daily soil temperature range: 22.7° to 33.3° C.

TREATMENT	AVERAGE GERMINATION[1]
	Percent
1 percent NH_4SCN 24 hours	[2]78.2
1 percent NH_4SCN 24 hours, wash	91.5
1 percent NH_4SCN 24 hours, dry 24 hours	86.2
Tap water 24 hours	81.2
Tap water 24 hours, dry 24 hours	53.5
Dry control	66.8

[1] Difference of 7.2 percent necessary for significance.
[2] Final stand was 67.3 percent, 10.9 percent drying after emergence.

When the thiocyanate-treated seed was either washed thoroughly with running water or dried before planting, the beneficial effect of the treatment on germination was again manifested (table 11). The germination obtained with

these treatments was about equal to the germination of the unwashed, undried thiocyanate-treated seed planted in used sterilized soil shown in table 9. The detrimental effect of planting the treated seed in freshly sterilized soil immediately after soaking is shown in two ways. The first effect was the reduction in emergence of the seedlings, and the second effect was the drying of the seedlings after emergence thus reducing the final stand as shown in table 11. This reduction in germination and stand also occurred in the previous experiments when freshly sterilized soil was used. The probability of the thiocyanate-treated seed being stored and still maintaining high germinative capacity is clearly demonstrated by the fact that whereas the thiocyanate-treated seed is still effective even after drying for 24 hours, the water-soaked seed seems to drop in germinative power even after 24 hours of drying. How long the thiocyanate-treated seed will maintain its original germinative capacity in dry storage remains to be determined. Soil temperature also is influential in the NH_4SCN treatment. Soil temperatures above 34° C. seemed to reduce the beneficial effect of the treatment as seen in table 10.

Thus if ammonia in the soil were the limiting factor, then it would seem that an excessive amount of it is detrimental to the germination of *Pennisetum setosum* seed. The beneficial effect of NH_4SCN under favorable temperature conditions is probably due to its favorable action on the respiration reaction.

Germination in petri dishes

In a petri dish series, the effect of 0.5, 1 and 2 percent solutions of NH_4SCN on germination of seed of *Pennisetum setosum* continuously germinated in the solutions, and of seed soaked in the solutions prior to germinating and then transferred to tap water substratum was determined. It was found that all concentrations of the solution were detrimental to germination, and that germi-

TABLE 12. Effect of temperature on germination of thiocyanate-treated seed of *Pennisetum setosum*.

Replications: 4 (100 seeds each).
Germination in petri dishes.
Germination period: June 14, 1940, to July 12, 1940.

GERMINATION TEMPERATURE	TREATMENT	GERMINATION DURING SOAKING	GERMINATION AFTER 28 DAYS	TOTAL GERMINATION
		Percent	*Percent*	*Percent*
7.8° C.	1 percent NH_4SCN	0.0	38.5	38.5
	Tap water	51.0	7.2	58.2
10.1° C.	1 percent NH_4SCN	0.0	48.0	48.0
	Tap water	53.0	15.2	68.2
15.2° C.	1 percent NH_4SCN	0.0	57.2	57.2
	Tap water	49.5	32.0	81.5
20.1° C.	1 percent NH_4SCN	0.0	89.2	89.2
	Tap water	51.2	38.0	89.2
25.2°—29.6° C. (room temperature)	1 percent NH_4SCN	0.0	96.0	96.0
	Tap water	44.8	34.5	79.3
31.2° C.	1 percent NH_4SCN	0.0	67.0	67.0
	Tap water	45.5	25.2	70.7

nation in tap water following soaking in 1 percent solution of the salt produced the highest germination (above 90 percent) at room temperature.

A series was conducted to determine the effect of different temperatures on the germination of thiocyanate-treated seed. The seeds were soaked in the solution at room temperature for 24 hours, then germinated in petri dishes with tap water at the various temperatures listed in table 12 in which are also tabulated the germination results.

That the germination of the seed of *Pennisetum setosum* is affected by temperature is clearly brought about by results presented in table 12. About half of the seeds soaked in tap water germinated during the soaking. This was probably the result of aging, since in the previous soil experiments when the seeds were fresher, only a few germinated during the soaking. None of the seeds in the thiocyanate solution germinated during the soaking, showing that there was a temporary inhibition. As the temperature increased, there was an increase in the percentage germination of both the thiocyanate-soaked and the water-soaked seeds up to a certain temperature beyond which the germination percentage fell. Thus the maximum germination of the thiocyanate-treated seed occurred at room temperature; of the water-soaked seed, at approximately 20° C.

A similar experiment was conducted using alternating temperatures to simulate the fluctuation in soil temperature. The seeds for the investigations dis-

TABLE 13. Germination of freshly harvested seed of *Pennisetum setosum* as affected by temperature and pre-treatment with NH_4SCN.

Replications: 4 (100 seeds each).
Germination in petri dishes.
Germination period: February 25, 1941, to April 1, 1941.

TREATMENT	AVERAGE TOTAL GERMINATION AT END OF —				
	1 week	2 weeks	3 weeks	4 weeks	5 weeks
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
Continuous room temperature (22.1°—26.1° C.):					
1 percent NH_4SCN 24 hours	1.0	58.0	68.8	74.6	.....
1 percent NH_4SCN 24 hours, wash	3.5	63.0	66.2	66.7	.....
1 percent NH_4SCN 24 hours, dry 48 hours	13.0	67.5	70.5	71.5	.....
Tap water 24 hours	0.8	0.8	0.8	[1]22.8	[2]22.8
Tap water 24 hours, dry 48 hours	0.5	1.5	3.7	[1]55.7	[2]55.7
Continuous germinator temperature (33° C.):					
1 percent NH_4SCN 24 hours	0.0	0.0	0.2	0.2	[3]52.4
1 percent NH_4SCN 24 hours, wash	0.2	0.2	0.2	0.2	[3]39.2
1 percent NH_4SCN 24 hours, dry 48 hours	0.2	0.4	0.6	0.6	.....
Tap water 24 hours	0.2	0.2	0.2	[1]4.2	[2]5.2
Tap water 24 hours, dry 48 hours	0.0	0.0	0.0	[1]8.0	[2]8.0
Alternating temperature (22.1°—25.8° C. and 33° C.):					
1 percent NH_4SCN 24 hours	3.0	61.5	83.7	87.9	.....
1 percent NH_4SCN 24 hours, wash	2.2	43.4	59.2	68.0	.....
1 percent NH_4SCN 24 hours, dry 48 hours	40.0	70.2	72.7	75.9	.....
Tap water 24 hours	0.2	0.2	0.2	[1]23.2	[2]23.2
Tap water 24 hours, dry 48 hours	0.0	0.5	0.5	[1]63.5	[2]63.5

[1] One week after cutting seed coat.
[2] After one week in 0.5 percent NH_4SCN substratum.
[3] After one week of alternating temperature conditions.

cussed up to this point were all from the same stock, but for this experiment a new supply of seed harvested locally on January 17, 1941 (100 percent normal seed) was used to determine the effect of the thiocyanate treatment on the germination of very fresh seed (the seed was used five weeks after harvest). The germination results of this experiment are tabulated in table 13.

The results in table 13 finally establish the fact that even with very fresh seed, the germination of thiocyanate-treated seed is affected by temperature. It is seen that constant high temperature is detrimental in all cases. Cutting of the seed coat of the ungerminated seeds of the tap water-soaked treatments at germinator temperature had no effect, nor did the shifting of the cut seed to a 0.5 percent thiocyanate substratum produce any effect on the final germination percentage. Subjecting the ungerminated seeds of the thiocyanate-treated seeds at the high temperature to alternating temperatures resulted in a marked increase in germination in one week. Although good germination was obtained at room temperature with the thiocyanate treatment, slightly better results were obtained with alternating temperatures, somewhat comparable to the temperature conditions which obtained in the soil series in which this treatment was responsible for increased germination. That the age of the seed affects germination is clearly shown in this experiment where very fresh seed soaked in tap water produced barely any germination under the temperature conditions used, in contrast to the previous soil and petri dish series in which much older seed was used and fair germination resulted. This fair germination was obtained when the ungerminated seeds, at room temperature and alternating temperatures, were cut (table 13); transferring the ungerminated cut seeds to a 0.5 percent thiocyanate substratum did not result in germination. As in the previous soil and petri dish series, whether the thiocyanate-treated seed was germinated without washing, after a thorough washing, or after drying, the chemical always had a favorable effect.

That the absorption of water by the seed is of no moment in the germination response produced by the thiocyanate treatment is shown by the fact that in one instance, the seed soaked for 24 hours in the solution absorbed 22 percent of its air-dry weight, while the seed soaked in pure tap water absorbed 24 percent of its air-dry weight.

Summary

The seed coat of *Pennisetum setosum* somewhat hinders the free movement of gases in and out of the seed, thereby reducing germination. Germination of this seed is also delayed by the lack of some respiratory and perhaps nutritional stimulant. Soaking the seed in water for 24 hours or altering the seed coat by cutting improves germination. Treating the seed with 1 percent solution of NH_4SCN for 24 hours in combination with room temperature or with alternating temperatures results in the best germination. Soaking in 0.5 percent vitamin C also increases the germination of this species, and so does soaking in 1 percent KNO_3 solution. The thiocyanate treatment is effective even after washing or drying, provided the temperature of germination is not too high and the germination medium does not contain ammonia in toxic amount.

In order to obtain good germination of this species in the field, the seed should be treated with ammonium thiocyanate and dried before planting. In the seed laboratory this and the other treatments may be used to obtain potential germination.

Panicum prolutum

Germination in soil

For the germination experiments on this species, several different lots of seeds harvested locally at different times were used. The percent normal seed was in all cases 100. The "seed" is naked with a rather hard glossy seed coat. The lots were composed of light-colored and dark-colored seeds, the former probably being immature seed. In all cases the two types were divided and treated separately, but because the response of these to the treatments used was about the same, the results obtained with the dark-colored seeds only are presented here.

In preliminary trials, the seed of *Panicum prolutum* did not respond to any chemical treatment. The germination percentage obtained as a result of pretreating with a chemical solution was no higher than that of the water-soaked control. In a replicated series, the effect of soaking the seed in water for various periods was determined, and the germination data are recorded in table 14.

TABLE 14. Soaking in tap water and in distilled water to increase the germination of the seed of *Panicum prolutum*.

Replications: 12 (50 seeds each).
Seed harvested October 7, 1939.
Germination in freshly sterilized soil.
Germination period: November 7, 1939, to December 5, 1939.

TREATMENT	AVERAGE GERMINATION[1]
	Percent
Tap water 6 hours	1.5
Tap water 12 hours	1.5
Tap water 24 hours	13.2
Tap water 48 hours	25.0
Tap water 72 hours	39.3
Distilled water 6 hours	1.7
Distilled water 12 hours	3.8
Distilled water 24 hours	14.8
Distilled water 48 hours	28.2
Distilled water 72 hours	17.7
Control	0.2

[1] Difference of 4.3 percent necessary for significance.

A progressive increase in germination occurred with the increase in soaking period both in tap water and in distilled water as shown in table 14. The 39 percent germination of the 72-hour tap-water soaking period is very high compared to the control. The increase in germination was about equal for the tap water and distilled water treatments in all cases except one—the 72-hour soaking treatment in which the seeds soaked in distilled water for 72 hours dropped in germination below that of the 48-hour soaking period.

With a second lot of seed harvested on February 15, 1940, the above experiment was repeated on March 25, 1940, using only tap water. This time there was no response to the soaking treatments. At about the same time, an acid scarification experiment was performed using the same lot of seed. The germination results of this series are recorded in table 15.

The effect of acid scarification on the germination of *Panicum prolutum* seed which did not respond to the water-soaking treatment is clearly shown in table 15. Because age of the seed might have been responsible for this lack of response, the seed was stored at room temperature for 5½ months. At the

TABLE 15. Effect of acid scarification on germination of *Panicum prolutum* seed.

Replications: 12 (50 seeds each).
Seed harvested February 15, 1940.
Germination in freshly sterilized soil.
Germination period: March 22, 1940, to April 19, 1940.

TREATMENT	AVERAGE GERMINATION[1]
	Percent
Concentrated H_2SO_4 3 minutes	7.0
Concentrated H_2SO_4 6 minutes	18.3
Concentrated H_2SO_4 10 minutes	36.7
Concentrated H_2SO_4 12 minutes	35.2
Concentrated H_2SO_4 20 minutes	39.5
Control	0.2

[1] Difference of 5.6 percent necessary for significance.

end of this time, as shown by a preliminary experiment, the seeds responded definitely to the soaking-in-water treatment. A replicated series using the water-soaking treatment and acid treatment was then conducted. Table 16 records the results.

TABLE 16. Effect of soaking in tap water and of acid scarification on the germination of seed of *Panicum prolutum*.

Replications: 6 (50 seeds each).
Seed harvested February 14, 1940.
Germination in freshly sterilized soil.
Germination period: July 29, 1940, to August 26, 1940.
Average daily soil temperature range: 24.5° to 35.1° C.

TREATMENT	AVERAGE GERMINATION[1]
	Percent
Tap water 1 day	43.4
Tap water 2 days	41.6
Tap water 3 days	44.7
Tap water 4 days	49.0
Tap water 5 days	40.0
Tap water 6 days	37.0
Tap water 3 days, dry 3 days	11.3
Tap water 3 days, dry 3 days, tap water 1 day	31.0
Tap water 3 days, dry 3 days, tap water 2 days	39.0
Tap water 3 days, dry 3 days, tap water 3 days	46.4
Concentrated H_2SO_4 5 minutes	27.4
Concentrated H_2SO_4 10 minutes	57.4
Concentrated H_2SO_4 10 minutes, dry 24 hours	51.4
Concentrated H_2SO_4 15 minutes	44.7
Concentrated H_2SO_4 20 minutes	17.7
Concentrated H_2SO_4 25 minutes	2.3
Concentrated H_2SO_4 30 minutes	1.7
Dry control	6.7

[1] Difference of 9.0 percent necessary for significance.

Table 16 clearly indicates that age of the seed influences reaction to the water-soaking treatment. Five weeks after harvest, the seed did not respond to this treatment, but approximately 5½ months after harvest, responded as shown in table 16. Acid scarification caused response as in the previous case (table 15). Length of the period of soaking in water had no effect on the resulting germination, but some seeds in the longer soaking periods (three days and longer) germinated during soaking. This places the longer soaking periods

in a rather disadvantageous position from the standpoint of practical application. Drying water-soaked seed before planting lowered germination to the level of the dry control. However, if the dried seed were resoaked in tap water, the germination of the corresponding original soaking was recovered. The water-soaking treatment was about as effective as the best acid scarification treatment. Drying of the acid-treated seed for 24 hours before planting did not affect the germinative capacity of the treated seed.

In another series, a lot of seed harvested on June 17, 1940, was given the acid treatment in combination with NH_4SCN and tap water treatments. At the time the experiment was conducted, the seed was two months old. The results showed that the thiocyanate was ineffective in promoting germination. However, soaking the acid-treated seed in tap water for 24 hours materially increased germination. The following relationship is significant: Dry control seed germinated only 2.0 percent; soaking in tap water for three days produced a germination of 37.7 percent; 10-minute acid treatment produced 48.7 percent; and 10-minute acid plus 24-hour water soaking resulted in a germination of 62.7 percent. Again it was demonstrated that drying of acid-treated seed had no detrimental effect on germination.

In a final replicated series using another lot of seed, the effect of KNO_3 and "Vita-flor" in combination with acid and water-soaking treatments was studied. As in the case of NH_4SCN, KNO_3 and "Vita-flor" were found to be ineffective in promoting the germination of *Panicum prolutum* seed. Here again, as in the previous experiment, the effect of soaking in water, the effect of acid scarification, and the effect of acid scarification plus water soaking were clearly demonstrated.

The acid-treated seed used in this experiment when stored dry at room temperature produced a germination of 32.0 percent after a storage period of 19 days as compared with the initial germination of 36.0 percent. After a storage period of 50 days, germination dropped to 16.7 percent. The control lot each time germinated less than 1 percent. Acid-treated black locust seed was found by Meginnis (35) to keep for a considerable period without losing its vitality if stored perfectly dry.

Since modification of the seed coat favors germination and salt absorption does not, it is apparent that an external seed factor rather than an internal condition of the seed is responsible for germination of *Panicum prolutum* seed. Furthermore, this external condition is modified by the age of the seed so that newly harvested seed does not respond to the water-soaking treatment as much as older seed.

Germination in petri dishes

Examination of the seed of *Panicum prolutum* indicates no reason why all of the seed should not germinate. From all outward appearances each seed is mature, uninjured, and normal. Despite this, the maximum germination obtained has been approximately 60 percent. Variation in thickness of the seed coat was suspected of influencing germination. This interpretation was tested in an experiment in which seeds were first germinated in tap water in petri dishes at room temperature. After two weeks, the ungerminated seeds, after being dried thoroughly at room temperature, were treated with concentrated H_2SO_4 for five minutes, washed thoroughly with water, and germinated again in petri dishes. Seeds that did not germinate within two weeks were again dried, treated with acid for two minutes, washed and germinated. Another

1-minute acid treatment was given, and finally the seed coats were cut, at which time all of the seeds either germinated or died. The percent germination and the percent injury owing to treatment are recorded in table 17.

TABLE 17. Effect of variation in thickness of seed coat on germination of seed of *Panicum prolutum*.

Replications: 12 (100 seeds each).
Seed harvested October 25, 1940.
Germination in petri dishes.
Germination period: December 26, 1940, to February 26, 1941.
Room temperature range: 21.2° to 26.3° C.

TREATMENT NUMBER (Successive treatments)	AVERAGE GERMINATION	AVERAGE INJURY
	Percent	*Percent*
1. None	[1]0.5	[1]0.0
2. Concentrated H_2SO_4 5 minutes	[1]61.1	[1]12.2
3. Concentrated H_2SO_4 2 minutes	[2]8.8	[2]11.8
4. Concentrated H_2SO_4 1 minute	[2]0.3	[2]4.1
5. Seed coat cut	[2]0.0	[2]1.2
Total	70.7	29.3

[1] Germination period 2 weeks.
[2] Germination period 1 week.

That there was variation in thickness of the seed coat of *Panicum prolutum* seed was demonstrated in this experiment as shown by the results recorded in table 17. Seeds with thin coats were affected by a short period of acid treatment, and those with thicker coats were affected by a longer period of acid scarification. Injury occurred whenever the acid treatment was too severe for the coat.

Since the seed coat seemed to be the factor determining the germination of the seed of this species, an attempt was made to determine the role it was playing. Was the seed coat preventing the absorption of water? Was it preventing the emergence of the embryo, or was it hindering the gaseous exchange necessary in respiration?

The first question was investigated. A lot of seed was treated with acid, dried thoroughly, weighed, and soaked in water for a given length of time. Excess moisture was removed on paper towelling, and the seeds were weighed again. Untreated seed was also soaked in water. After soaking for 24 hours in water, the acid-treated seed absorbed moisture to 62.7 percent of its air-dry weight and the untreated absorbed 58.6 percent of its air-dry weight. Similar results were obtained in other trials. Both the acid-treated and untreated seeds were fully swollen after soaking. Thus it is seen that the seed coat of normal seeds does not prevent the intake of water.

An acid-treatment of 12 minutes resulted in 75 percent germination at room temperature as compared with 6 percent in the control. The seed coats of the ungerminated seeds of the control were cut, weighed without drying, and soaked in water for 24 hours at the end of which period, it was shown that these seeds did not absorb any additional water. This is further proof of the permeable nature of the seed coat and shows that the cause of low germination is not imperviousness of the seed coat.

The possibility of the seed coat preventing the emergence of the developing embryo was determined. In all cases it was noticed that with the appearance

of the primary root and shoot, the seed coat almost invariably split along the lateral ridges where the two parts of the coat (one part covering the dorsal or germ side of the seed and the other covering the ventral or endosperm side of the seed) came together. It was thought that perhaps a cut made along the ridges would help in the emergence of the young embryo and that a cut made in a position removed from the ridges would not promote germination. Examinations of embryos of ungerminated seeds in germinative medium showed no embryos which had started to grow, although they had fully imbibed.

Some ungerminated seeds of a control lot of an experiment were cut along the ridges and some were cut cross-wise midway between the embryo and the end of the seed on the dorsal side. The cuts were made without injuring the endosperm. The result was that the seeds with the ridges cut germinated 99 percent in 6 days, while those with the cross-cut resulted in germination of 97 percent in 12 days. It was found in later experiments that no matter where the cut was made on the seed coat, provided the embryo was not injured, the treatment promoted germination. Regardless of the position of the cut, the seed coat in nearly all cases split along the ridges as the young embryo emerged. It does not seem that the seed coat is imprisoning the young embryo, but rather that some factor for the initiation of embryo growth is lacking. That factor seems to be gaseous exchange.

To test whether an increased amount of dissolved oxygen in the germination medium would influence germination, seeds were germinated in 1, 3, and 30 percent H_2O_2 at room temperature. There was no response to these treatments. Soaking the seed in ether for four minutes did not increase germination.

The effect of temperature on germination was studied. Some seeds were germinated in water substratum at 4° C., and some at 36° C. In one week no germination occurred at the low temperature, but 1 percent germination occurred at the high temperature. Then the seeds at the high temperature were subjected to the cold temperature, and *vice versa.* The results were that seeds shifted from the low to the high temperature germinated 23 percent in one week, whereas those shifted from the high to the low temperature produced no additional germination. Then the high temperature was lowered to 33° C., but the low temperature was maintained. Under these conditions, the low-to-high change produced no additional germination, but the high-to-low change resulted in germination of 7 percent in one week. Then the seeds were subjected to 24-hour alternating (24-hour high, 24-hour low) temperatures. In one week the seeds originally in the cold germinated 6 percent, but those originally in the high temperature produced no germination. Then the 24-hour alternation was changed to 6-hour high temperature and 18-hour low temperature alternation daily. In one week under this alternation, the seeds originally in the low temperature germinated 2 percent, and those originally in the high temperature germinated an additional 11 percent. The subsequent treatments of 4° and 36° C. daily alternation, room temperature and 36° C. daily alternation, and cutting the seed coat produced no additional germination for the two lots of seed. By this time the seeds were believed dead. The total germination for the seeds originally at the low temperature was 31 percent, and for those originally at the high temperature the germination was 19 percent.

In another experiment, the seeds were subjected to alternating temperatures and germinated in a substratum of 0.2 percent KNO_3 as a possible stimulant. The temperature treatments and germination results are recorded in table 18.

TABLE 18. Alternating temperatures (6 hours at high and 18 hours at low daily) as affecting germination of *Panicum prolutum* seed.

75 seeds each treatment.
Seed harvested October 25, 1940.
Germination in petri dishes.
Germination period: March 6, 1941, to March 27, 1941.

TREATMENT	GERMINATION IN 3 WEEKS
	Percent
0.2 percent KNO_3 substratum:	
4° C. and room temperature[1]	0.0
4° C. and 33° C.	17.3
4° C. and 36° C.	8.0
Tap water substratum:	
4° C. and room temperature[1]	0.0
4° C. and 33° C.	26.5
4° C. and 36° C.	16.0

[1] 22.3° to 26.1° C.

Table 18 shows that, as in the previous experiment, temperature alternation is somewhat beneficial to germination. A daily alternation of 4° to 33° C. seems to be the best, room temperature being too low and 36° C. being too high for the higher temperature in the alternation. Tap water substratum is superior to 0.2 percent KNO_3 substratum.

The increased germination caused by the change from a cold to a high temperature was probably due to the release of trapped carbon dioxide gas as a result of its lessened solubility in the seed solution at the higher temperature.

As a further proof of the role the seed coat plays in the gaseous exchange, the following two experiments may be considered. In the first, dried acid-treated seeds (12-minute treatment) and seeds with cut seed coats were placed in petri dishes with tap water as substratum and the whole placed in a desiccator and subjected to a partial vacuum of approximately 25 mm. pressure. In one week, no germination occurred at room temperature in either lot of seed, although the seeds were fully swollen and split along the lateral ridges of the seed coat. When the seeds were exposed to full atmospheric pressure, germination in one week was 93 and 88 percent for the acid-treated and cut seeds, respectively.

In the second experiment, the seed coats of some ungerminated seeds that had been soaked in tap water at room temperature for 45 days producing only 6 percent germination during that period, were cut, and the seeds put in a desiccator and subjected to a partial vacuum of approximately 25 mm. pressure. In addition, a similar lot was germinated at full atmospheric pressure. In one week, at room temperature, the seeds in the partial vacuum germinated 17.0 percent, while those in the full atmosphere germinated 38.3 percent. When about one-fourth atmospheric pressure was let into the vacuum, an additional germination of 57.4 percent occurred in one week, and those in the full atmosphere increased their initial germination by 17.0 percent. When the seeds in the one-fourth atmospheric pressure were exposed to the full atmosphere, an additional germination of 14.9 percent occurred in one week, and the seeds in the full atmosphere from the beginning germinated an additional 8.5 percent. The seeds in the full atmosphere from the beginning were then subjected to a partial vacuum for two days and then given the full atmospheric pressure. The result was that an additional 8.4 percent germinated in two weeks. The other treatment increased germination by 6.3 percent during this period.

Germination totaled 95.6 percent for the seeds originally in partial vacuum and 72.2 percent for those originally in the full atmospheric pressure. Of particular interest here is the fact that the seeds in the partial vacuum germinated, whereas in the previous experiment, they did not. The only difference between the two experiments is that in the previous case, dry seeds were used, whereas in the present case, seeds soaked for a prolonged period were used. The explanation is probably that in the soaked seed, CO_2 accumulation was faster than in the dry seed because of the increased respiratory activity (though very low) caused by the absorption of more oxygen in the soaked seed than in the dry seed. When the trapped CO_2 in the soaked seed was removed by reducing pressure through the cut made in the seed coat, germination resulted even in the near absence of oxygen. The difference in the final germination between the two treatments in the present experiment was probably due to the initial fast elimination by the vacuum of the CO_2 trapped in the seed. This method of elimination of CO_2 produces an increase in germination similar to that obtained when the gas is released at high temperature.

Summary

Low germination of the seed of *Panicum prolutum* is determined by the character of the seed coat which normally is not permeable to gas. When this impermeable nature is altered by soaking the seed in water for 1 to 3 days (for older seed), by scarifying with concentrated H_2SO_4 for 10 to 15 minutes (for fresh seed), by cutting the seed coat, or by subjecting the seed to extreme daily alternating temperatures of 4° and 33° C., germination is improved. Combined acid and soaking-in-water treatments also promote the germination of this species. Dried acid-treated seed retains its viability and effectiveness for some time.

Increased germination of this species in the field can be obtained by presoaking the seed in water (for old seed) and by sulphuric acid scarification (for fresh seed) before planting. For laboratory germination tests these and the other methods can be employed.

Cenchrus biflorus

Germination in soil

Germination investigations on this species were conducted on several different lots of seed harvested locally at different times. The seeds of this species are enclosed in a bur. Each bur consists of 1 to 5 spikelets clumped together, and the number of caryopses in each bur ranges from none to five. All germination percentages were based on the number of normal caryopses in a given lot of seed.

Preliminary trials with various temperature treatments, compressed air and oxygen, nutrient solutions, and various chemicals (ethylene chlorhydrin, ethyl alcohol, NaSCN, $(NH_2)_2CS$, 1-leucine, heteroauxin, H_2O_2, etc.) failed to improve the germination of seeds of this species. Only mechanical scarification was effective in improving germination; sometimes acid scarification was effective.

In one series the following germination results were obtained in soil: sand paper scarification, 29.4 percent; scarification with food chopper, 9.5 percent; seed coat cut, 37.5 percent; and control, 5.9 percent. The low germination of these mechanically scarified seeds was the result of injury sustained by the embryos in the scarification process. Two factors that made scarification dif-

ficult were the presence of the seeds in burs and the lack of uniformity in the size of the caryopses.

In another experiment with another lot of seed, scarification with concentrated H_2SO_4 for two minutes resulted in germination of 55.0 percent. The control germinated 35.5 percent. In most instances, however, acid scarification was detrimental to germination.

Germination in petri dishes

A number of experiments were conducted in petri dishes at room temperature to determine the cause of dormancy of the seed of *Cenchrus biflorus*. As in the soil series the seeds with their seed coats cut produced good germination. In one series the naked seed germinated 7 percent; the naked seed with seed coat cut, 76 percent; and the control, 0 percent. The same treatments when used on other lots of seed produced similar results. In all cases the treated and untreated seeds freely absorbed water from the germination medium.

Thus it seems that the seed coat of *Cenchrus biflorus* like that of *Panicum prolutum* hinders the passage of gases. The small increase in germination of the uncut naked seeds was probably due to a slight injury sustained by some of the seed coats in the process of removing them from the burs. When the seed coat was cut or scarified without injury to the embryo, gas exchange was facilitated, and the seed germinated.

Summary

The germination of the seed of *Cenchrus biflorus* is delayed by the presence of a seed coat which hinders gas exchange. Modification of the seed coat by mechanical scarification promotes gas exchange and induces germination.

There are no adequate practical means of scarifying the seed of this species without injuring the embryo, but with some care and patience, the seed may be scarified effectively by rubbing the burs between layers of sand paper. The seed analyst may cut the seed coat to obtain maximum germination.

Paspalum notatum

Germination in soil

The seeds of *Paspalum notatum* used in the following experiments were of several different lots harvested locally at different times. The percent normal seed varied from 30 to 60.

In several small replicated trials, it was shown that scarification with concentrated H_2SO_4 increased germination of the seed of this species in soil. A large replicated experiment in which the time of treatment with the acid was varied was conducted. The results of this experiment are recorded in table 19.

The effect of acid scarification on the germination of the seed of *Paspalum notatum* is very marked (table 19). A progressive increase in germination occurred with an increase in the duration of treatment to the 30- and 35-minute periods when the maximum germination was more than 70 percent which compares very favorably with the less than 1 percent germination of the control. Beyond these periods, a progressive detrimental effect set in as treatment was prolonged.

A similar experiment was conducted with another lot of seed. A soaking-in-tap-water treatment was also included in this experiment. The highly beneficial effect of the acid scarification was again demonstrated, the 35-minute treatment (optimum) producing a germination of 85.5 percent and the untreated 4.8 percent. Soaking in tap water produced no effect.

TABLE 19. Effect of acid scarification on the germination of *Paspalum notatum* seed.

Replications: 10 (100 seeds each).
Seed harvested October 17, 1939.
Percent normal seed: 49.
Germination in freshly sterilized soil.
Germination period: December 6, 1939, to January 3, 1940.

TREATMENT	AVERAGE GERMINATION[1]
	Percent
Concentrated H_2SO_4 3 minutes	7.6
Concentrated H_2SO_4 5 minutes	19.8
Concentrated H_2SO_4 10 minutes	22.6
Concentrated H_2SO_4 20 minutes	28.8
Concentrated H_2SO_4 30 minutes	72.0
Concentrated H_2SO_4 35 minutes	73.5
Concentrated H_2SO_4 40 minutes	44.0
Concentrated H_2SO_4 45 minutes	31.2
Concentrated H_2SO_4 60 minutes	9.0
Concentrated H_2SO_4 75 minutes	3.2
Control	0.2

[1] Difference of 13.4 percent necessary for significance.

In another experiment, the acid-treated seeds were either dried and soaked in 1 percent KNO_3 and 1 percent NH_4SCN solutions before planting or they were soaked in the solutions without drying. Seeds treated thus were either planted after drying or without drying. The results showed that the nitrate and thiocyanate treatments did not increase germination above the already increased germination of the acid-treated seed. Nor did the water-soaking have any effect after the acid treatment. Mere drying of the acid-treated seed before planting, did not, however, reduce the germination of the acid-treated seed planted immediately after the treatment.

An experiment was conducted to verify the last two observations given above, and the results are recorded in table 20.

The results of the last experiment presented in table 20 show definitely that unlike the seed of *Panicum prolutum* which responds to water-soaking after the acid treatment, the seed of *Paspalum notatum* is not affected by the after-soaking in water and that like the seed of *Panicum prolutum,* drying of the acid-treated seed does not reduce germination of the treated seed. This difference in the behavior of the acid-treated seeds of these two species is due to different types of inhibition to germination as will be shown in the petri dish experiments.

Summarizing the results presented above, it is seen that maximum germination of *Paspalum notatum* seed in soil is obtained by treating the seed with concentrated H_2SO_4 for 30 to 40 minutes and planting either dried or not dried. Burton (8), using the same treatment on this species, obtained germination of 57 percent in soil with a 10-minute treatment.

Germination in petri dishes

A description of *Paspalum notatum* seed is pertinent here. The "seed" is composed of a caryopsis enclosed in a chamber formed by a thick and tough fertile lemma on the dorsal (germ) side and by an equally tough palea on the ventral side. The lemma has its edges inrolled very tightly around the palea edges along the lateral ridges of the caryopsis. Outside the palea is the thin

TABLE 20. Effect on germination of *Paspalum notatum* seed of drying and of soaking in water after acid scarification.

Replications: 6 (150 seeds each).
Seed harvested October 16, 1940.
Percent normal seed: 30.
Germination in used sterilized soil.
Germination period: December 11, 1940, to January 8, 1941.
Average daily soil temperature range: 20.8° to 29.9° C.

TREATMENT	AVERAGE GERMINATION[1]
	Percent
Concentrated H_2SO_4 25 minutes	45.2
Concentrated H_2SO_4 32 minutes	55.9
Concentrated H_2SO_4 35 minutes	52.2
Concentrated H_2SO_4 40 minutes	64.0
Concentrated H_2SO_4 25 minutes, dry 24 hours	50.4
Concentrated H_2SO_4 32 minutes, dry 24 hours	56.3
Concentrated H_2SO_4 35 minutes, dry 24 hours	47.4
Concentrated H_2SO_4 40 minutes, dry 24 hours	60.0
Concentrated H_2SO_4 32 minutes, tap water 24 hours	58.5
Concentrated H_2SO_4 32 minutes, tap water 24 hours, dry 24 hours	63.0
Concentrated H_2SO_4 32 minutes, dry 24 hours, tap water 24 hours	60.4
Concentrated H_2SO_4 32 minutes, dry 24 hours, tap water 24 hours, dry 24 hours	40.4
Tap water 24 hours	0.0
Dry control	0.0

[1] Difference of 16.1 percent necessary for significance.

sterile lemma, and outside the lemma is the equally thin second glume. Both of these structures are easily detached in dry and soaked seed and hence play no part in the mechanics involved in the germination behavior of this seed. The term "hull" as used here refers to the structures enclosing the caryopsis.

In one of the attempts to determine the absorption of water by acid-treated and untreated seeds, it was observed that the hard lemma and palea did not prevent the absorption of water by the caryopsis of the untreated seed. Actually as determined by weight, the acid-treated (32 minutes) seed absorbed 2½ times more water in 48 hours than did the untreated seed. This includes the absorption of water by the hulls which become swollen in soaking, and the acid-treated seed, of course, had much less hull left on the seed as the result of treatment. Careful examination of the acid-treated and the untreated seed after the soaking presented an interesting situation. The caryopsis of the acid-treated seed with a large proportion of hull removed was much more swollen than the caryopsis of the untreated seed. This resulted from a situation where in one case the caryopsis and the germ in particular were able to expand to the maximum capacity, whereas in the other case, were limited in their expansion by the strong unexpanding walls around them. In a dry seed, the caryopsis lies more or less loosely within the hull, but in an untreated soaked seed, it is pressed against the interior of the hull. Furthermore, the caryopsis of a dry untreated seed is hard and brittle, whereas the caryopsis of an untreated seed after a period of soaking in water is soft. The embryos of such soaked seed give no evidence of starting growth. Thus from the above considerations, it is seen that the inhibition of germination of untreated seed reported in the soil

series was very likely due to the interference of the lemma and palea with the maximum imbibitional expansion of the embryo and the caryopsis in general. The following experiments will bear out this point.

In the first experiment, acid-treated (32 minutes with concentrated H_2SO_4) and untreated seeds soaked in water for the absorption test were germinated at room temperature after the test. The acid-treated seed germinated 75 percent in 11 days, but the untreated seed did not germinate. The ungerminated seeds of the untreated lot were subjected to the various additional treatments, such as cutting the hull, removing the lemma and palea, and germinating in vacuum listed in table 21. This table also gives the germination results of each treatment.

TABLE 21. Effect of cutting and of removing the lemma and palea on germination of *Paspalum notatum* seed under various atmospheric conditions.

10 to 50 normal seeds per treatment.
Seed harvested October 16, 1940.
Germination in petri dishes.
Germination period: March 26, 1941, to April 16, 1941.
Average daily room temperature range: 22.5° to 26.8° C.

SEED TREATMENT	SUCCESSIVE ATMOSPHERIC CONDITIONS OF GERMINATION AND ADDITIONAL SEED TREATMENTS	CUMULATIVE GERMINATION IN SUCCESSIVE 7-DAY PERIODS
		Percent
Naked[1]	Vacuum (25 mm.)	0.0
	↓	
	One-half atmosphere	20.0
	↓	
	Full atmosphere	50.0
	Full atmosphere	70.0
	↓	
	Full atmosphere	80.0
	↓	
	Full atmosphere	90.0
Cut[2]	Vacuum (25 mm.)	0.0
	↓	
	One-half atmosphere	0.0
	↓	
	Full atmosphere	0.0
	Full atmosphere:	
	Lemma removed	70.0
	Palea removed	0.0
	Naked[1]	100.0
	Lemma edges removed[3]	0.0
	Full atmosphere	0.0
	↓	
	Full atmosphere	0.0
	Full atmosphere:	
	Naked[1]	72.7
	Naked[1], seed end cut	90.9
	Lemma edges removed[3]	0.0
	Cut deeper[4]	0.0

[1] Lemma and palea removed.
[2] Brush end of hull cut off exposing caryopsis.
[3] Inrolled lemma edges removed thus separating lemma from palea.
[4] Brush end of hull cut deeper into the seed proper thus cutting away part of the caryopsis.

Because of the difficulty of handling the seeds and the tedious operations involved in removing the tough hulls, few seeds were used for each of the additional treatments. In spite of the small number used, the results obtained are highly significant. In the first place, the effect of the acid treatment is very real. Although 100 percent germination is possible, a 75 percent germination was obtained with the 32-minute treatment. The other 25 percent was injured by the treatment because of the variation in thickness and hardness of the hull. In the second place, the result obtained with the acid could be obtained by other modifications of the hull.

In the attempts made to germinate the ungerminated seeds of the original control lot, the first important factor is the necessity of oxygen for germination. Naked seeds (lemma and palea removed) did not germinate in partial vacuum; with the increase in the amount of air in the germinating chamber germination increased. In the full atmosphere, however, the naked seeds germinated immediately.

The second factor of importance is that the seed may obtain this necessary oxygen and yet not germinate as shown by the failure of the seeds to germinate in full atmosphere in the case of the seeds cut off at the brush end of the hull thus exposing the caryopsis within. If additional absorption of water were all that was needed for germination, the cut seeds certainly were in a position to absorb all the water they needed. If it were a matter of the seed coat and not the hull being impermeable to water and gas (this of course is not true as evidenced by the excellent germination of the naked seed), the seeds with part of the caryopsis and hull cut away were certainly in a position to absorb all the water and gas they needed when germinated in the full atmosphere, but they did not germinate under such conditions.

The third important factor is that, aside from acid scarification, the only way to make the seed germinate is to remove part or all of the hull from the caryopsis. Thus if the lemma only were removed, good germination occurred, and if the lemma and palea were removed excellent germination occurred. On the other hand, if only the palea were removed or if only the inrolled lemma edges were removed, no germination occurred even in full atmosphere. The cause of this difference in response, considering the fact that all of these treatments had exposed the seeds to air and water was investigated.

Stripping the lemma and palea from the seed results in additional swelling in all directions just before the now much swollen embryo breaks through the seed coat. In the case of the seeds with only the lemma removed and the palea intact, the swelling takes place only in the direction of the lemma, and since the germ at least is free to expand, it germinates, but more slowly and at a lower rate than that for the naked seed. When only the palea is removed and the lemma left intact, the swelling takes place only in the direction of the palea, and since the germ is not capable of reaching its maximum swelling on account of the resistant lemma, no germination occurs. When the inrolled edges of the lemma are removed, the lemma and palea are separated from each other except at the base (germ end) of the seed where they are more or less closely and firmly attached to the sub-sessile rachilla. Even in this condition, the fullest expansion of the caryopsis is prevented by the strongly clasping lemma and palea, and so the germ does not emerge. In a few cases, it was observed that the germ had started to grow, but it seemed unable to push the lemma up or push itself through the tough structure. In the optimum acid scarification treatment, most of the hull is destroyed, and the maximum germination occurs

when the lemma area just above the germ is destroyed in addition to all-around scarification so that the germ is free to push its way out of the seed.

In the second experiment, where dry seeds were used, removal of the lemma only, resulted in a germination of 70 percent, while the naked seeds germinated 100 percent and the untreated 0 percent.

The possibility of an inhibitor in the hull interfering with the germination as with the seed of *Pennisetum ciliare* to be discussed next, was investigated. Some naked seeds were germinated in a tap water substratum, and some in tap water substratum with detached hulls added. The resulting germination in each case was excellent.

Thus from the above considerations, it seems very likely that the germination of the normal seed of *Paspalum notatum* is very greatly interfered with by a very tough hull which prevents the maximum imbibitional swelling of the caryopsis and which also imprisons the young embryo if it has started to grow. In this connection, it is of interest to note that Crocker and Davis (15) found a case of dormancy in the seed of *Alisma plantago* very much like the one described here, except that maximum swelling of the embryo was prevented by mechanical restraint of the embryo by the seed coat.

Summary

The cause of low germination in seed of *Paspalum notatum* is the presence of a tough hull (lemma and palea) enclosing the caryopsis and preventing maximum expansion of the embryo and the seed. Acid scarification for approximately 35 minutes, or removal of the hull, results in increased germination. Drying of the acid-treated seed does not affect germinative capacity.

Sulphuric acid scarification with subsequent drying before planting has its practical application. In the seed laboratory the seed may be treated with acid or the hull removed to increase the germination of this species.

Pennisetum ciliare

Germination in soil

Germination studies on the seed of *Pennisetum ciliare* in soil were conducted on seed lots harvested locally at different times. The number of normal seed varied from 81 to 140 sound caryopses in a given sample of 100 fascicles. The spikelets of this species are grouped together in a cluster called a fascicle which is surrounded by rather stiff bristles. The number of spikelets for each fascicle varies from 2 to 3 or more, and each fascicle contains from 0 to 3 caryopses, each capable of germinating. Germination percentages were based on the number of normal caryopses in a given sample of 100 fascicles.

Preliminary trials with freezing and thawing treatments did not improve the germination of seed of this species. On account of the structure of the seed (fascicle), it was thought that if the extraneous materials (hull) surrounding the caryopses were removed, improved germination would result. In subsequent experiments using concentrated H_2SO_4 scarification, the expected improved germination resulted. In one of these series, a 5-minute treatment resulted in a germination of approximately 53 percent, whereas the germination of the untreated seed was approximately 36 percent.

The germination results of another series in which caryopses with all extraneous materials, except the thin lemma and palea which enclose the caryopsis were removed, were germinated in addition to the acid-treated fascicles, are recorded in table 22.

TABLE 22. Effect of acid scarification on germination of *Pennisetum ciliare* seed.

Replications: 12 (50 fascicles each = 60 caryopses).
Seed harvested February 15, 1940.
Normal seed: 120 caryopses in 100 fascicles.
Germination in freshly sterilized soil.
Germination period: April 17, 1940, to May 15, 1940.

TREATMENT	AVERAGE GERMINATION[1]
	Percent
Concentrated H_2SO_4 2 minutes	46.0
Concentrated H_2SO_4 4 minutes	46.5
Concentrated H_2SO_4 5 minutes	52.4
Concentrated H_2SO_4 7 minutes	57.6
Concentrated H_2SO_4 10 minutes	58.6
Concentrated H_2SO_4 20 minutes	49.0
Concentrated H_2SO_4 30 minutes	33.4
No acid treatment, bristles removed	74.0
Control	40.5

[1] Difference of 7.3 percent necessary for significance.

A progressive increase, then a decrease, in germination resulted with increase in the acid scarification time according to table 22. A treatment time of 5 to 10 minutes produced the best results with scarified fascicles, but the removal of the extraneous materials around the fertile lemma resulted in the highest germination.

In another experiment the results of which are not presented here because of their similarity to those obtained in the preceding experiment, it was found that drying the acid-treated fascicles before planting resulted in no detrimental effect on germination.

Germination in petri dishes

For the germination trials in petri dishes reported here, the seeds were of the same lot harvested locally on January 17, 1941. The number of normal seed was 140 caryopses in 100 fascicles.

The effect of the acid treatment on the absorption of water by the seed was determined. Some seeds were treated with concentrated H_2SO_4 for five minutes, dried thoroughly, weighed, then soaked in water. Another lot was clipped (the bristles were clipped off with a pair of scissors so that the caryopses were visible), weighed, and soaked in water. This treatment was an attempt to imitate exposure of the caryopses by the action of the acid on the bristles and the other extraneous materials of the fascicles. In the third lot, naked seeds were soaked in water, the hulls comprising the fourth lot, and in the last lot untreated fascicles were soaked in water. After 48 hours of soaking, the first lot absorbed 106 percent of its air-dry weight in water; the second lot 104 percent, third lot 45 percent, fourth lot 54 percent, and the fifth lot 102 percent. These results show that the hulls and the naked caryopses absorbed about the same amount of moisture.

After the absorption test, the acid-treated, clipped, and untreated fascicles were germinated in petri dishes at room temperature along with the naked seeds. The results of this experiment are found in table 23, in which are also listed the subsequent secondary treatments employed to induce growth of the ungerminated seeds of the fascicles.

TABLE 23. Germination of *Pennisetum ciliare* seed as affected by acid scarification and removal of all or part of hulls.

Replications: 4 (36 fascicles each = 50 caryopses).
Germination in petri dishes.
Germination period: March 15, 1941, to April 9, 1941.
Average daily room temperature range: 22.7° to 27.0° C.; alternating temperatures: 21.9° to 24.8° C. and 33° C.

TREATMENT	TOTAL GERMINATION AT —		GERMINATION AT ROOM TEMPERATURE IN 7 DAYS AFTER SECONDARY TREATMENTS	
	Room temperature on 11th day	Alternating temperatures on 18th day		
	Percent	*Percent*		*Percent*
Concentrated H_2SO_4 5 minutes	14.5	15.5	25 original fascicles	0.0
			25 spikelets separated[1]	0.0
			25 seeds with lemma and palea only	0.0
			25 seeds naked	44.0
Clipped fascicles	11.0	11.0	25 original fascicles	0.0
			25 spikelets separated[1]	0.0
			25 seeds with lemma and palea only	4.0
			25 seeds naked	56.0
Naked seeds	91.5	91.5		
Control	1.5	2.0	25 original fascicles	4.0
			25 spikelets separated[1]	8.0
			25 seeds with lemma and palea only	0.0
			25 seeds naked	76.0

[1] Bristles left on spikelets after separation.

It is seen in table 23 that the seed did not respond to the acid treatment when germinated in petri dishes as much as it did in soil. The effect of the clipped fascicles was about the same as that of the acid-treated fascicles. Naked seeds produced better than 91 percent germination, while the control germinated very much more poorly than any control in the soil experiments. An additional period under alternating temperatures did not increase the germination of any of the treatments beyond that obtained at room temperature.

From the results of the secondary treatments imposed on the ungerminated fascicles, it is seen that if any part of the hull remains attached to the caryopsis, germination is depressed, but if all of the hull is removed, good germination occurs.

When the caryopses of the ungerminated fascicles were examined, they were found to be fully swollen; so, it was not water that the seed lacked. Furthermore, the acid treatment and the clipping caused part of the caryopsis to be visible through an opening in the fertile lemma. Through this opening gaseous exchange and water absorption could take place as well as through the thin lemma and palea which are visible and partly exposed; yet, the caryopsis did not germinate.

The possibility that the hulls prevent the embryo from germinating by mechanical restraint on the seed was discarded, because, in the first place, the caryopsis is not so tightly held within the bristles; in the second place, it did not germinate when freed of the bristles; and finally, the thin and loose lemma and palea certainly could not imprison the developing embryo within.

A suspicion that something from the hulls was inhibiting the germination of the seed of *Pennisetum ciliare* led to the following experiments.

In a preliminary test, some naked seeds were germinated, and others naked but with the removed lemma and palea and bristles included in petri dishes with tap water as substratum, were germinated at room temperature. Other seeds were germinated with only the lemma and palea intact, others with the brush end of lemma and palea clipped off, and others with lemma and palea intact but with removed bristles in the substratum. In all cases where extraneous materials, intact or removed, were in contact with the caryopses, there was a marked reduction in germination. The naked seeds germinated 60 percent, seeds with extraneous materials averaged 29 percent, and the untreated 0 percent in seven days. Removal of hulls from the substratum and from the seeds followed by thorough washing of the seeds, resulted in an increase of germination from 29 percent to 52 percent in a 5-day period. Seeds naked from the start of the test showed no further germination. Untreated seeds showed no germination during the whole test up to this point. When the seed coats of all ungerminated seeds were cut, the naked seeds increased their germination to 93 percent, while the others increased theirs to 76 percent in seven additional days. The untreated still did not germinate. There was no doubt that some inhibitor secreted by the hull was preventing the germination of the normal seed. When the source of the inhibitor was removed, germination was improved. Inhibition was partly due to the seed coat which probably prevented adequate exchange of gases, since when the seed coat was cut, almost perfect germination occurred. Thus, it seems that the reduction in germination is caused by some inhibitor present in the hulls and by the inability of the seed coat to permit sufficient gaseous exchange.

A replicated series involving most of the treatments employed in the above experiment was conducted, and the germination results as recorded in table 24 show a very similar reaction of the hull material in inhibiting germination.

TABLE 24. Effect of the presence of removed and intact hulls on the germination of *Pennisetum ciliare* seed.

Replications: 3 (40 caryopses each = 29 fascicles).
Germination in petri dishes.
Germination period: April 9, 1941, to April 27, 1941.
Average room temperature range: 23.1° to 28.4° C.

TREATMENT	CUMULATIVE GERMINATION		
	Original 7th day	Hulls removed; seeds washed[2] 13th day	Ungerminated seeds cut[3] 18th day
	Percent	*Percent*	*Percent*
Naked caryopsis only	74.2	80.0	88.3
Naked caryopsis + removed lemma and palea	27.5	45.8	77.4
Naked caryopsis + removed bristles[1]	36.7	61.7	84.2
Naked caryopsis + removed lemma and palea + bristles[1]	25.8	54.1	83.3
Lemma and palea only on seed	23.3	56.7	70.0
Control	0.0	0.0	0.0

[1] Including glumes and sterile lemma.
[2] Naked caryopsis only and control left intact.
[3] Control left intact.

It will be remembered that in the soil series the control lot always germinated from 30 to 40 percent in four weeks. When some seeds of the present lot were planted in soil, the resulting germination was about 39 percent in three weeks, but in petri dishes it was near 0 percent. Considering the results of the above two experiments and the fair germination obtained with the control lot in soil, it is believed that in soil, some of the inhibitor is either fixed by the soil or washed out of the soil by water. The increase in germination caused by the acid treatment was probably the result of reduction in the amount of the inhibitor-producing material by the scarifying action of the acid.

Attempts were made to determine the nature of the inhibitor. Some naked seeds were germinated in tap water and some in a broth of cooked hulls. After one week the seeds in tap water germinated 90 percent, while those in the broth germinated only 48.9 percent. (The higher initial germination of the treatments in this experiment than in the previous experiments was due probably to the scarifying action of rubbing the seeds between the fingers in removing the lemma and palea; in the previous experiments, these structures were removed carefully with teasing needles without injuring the caryopses.) When tap water was substituted for the broth, the total germination increased to 67.9 percent in two additional days. The seeds in tap water from the beginning did not germinate any more during this period. When the seed coats of the ungerminated seeds were cut, those originally in the cooked hull broth increased their germination to 84.6 percent, while those in tap water from the beginning increased their germination to 92.2 percent (only 2.2 percent increase). Thus it seems that the inhibitor is some substance that is heat stable and non-volatile in tap water. Ammonia (50, 51) probably would have volatilized (pH of tap water is approximately 8), and most enzymes would be destroyed by boiling temperature. Tests for the liberation of free ammonia from the hulls under germinative conditions gave negative results. Molds which develop in the normal hulls when the seeds are subjected to germinative conditions, probably have no effect on germination, since cooked hulls which do not develop molds also inhibit germination.

In subsequent germination trials, naked seeds were germinated in filtered and unfiltered water extract of the cooked hulls, in water with residue of the cooked hulls (after filtering), and in water with thoroughly washed cooked hulls. The results showed no inhibition except in the last case where the hulls were present.

In further trials, naked seeds were germinated in water with hulls that had been soaked in cold ether and in cold and boiling 95 percent alcohol. The inhibitor was still active in reducing germination even after these treatments. Subjecting intact fascicles to running water did not increase germination. Suspending powdered charcoal above the fascicles in a petri dish was ineffective in improving germination. The result obtained in germinating intact fascicles in powdered charcoal in the laboratory was similar to that obtained in soil in the greenhouse.

Since Laibach and Keil (31) found that HCN was given off by germinating seeds, tests for cyanide were made on the hulls of *Pennisetum ciliare* seed. All tests gave negative results.

By cooking the hulls in dilute H_2SO_4 (3N and 6N), it was possible to inactivate the inhibitor.

From the above considerations, it seems that the inhibitor concerned in the reduction in germination of the seed of *Pennisetum ciliare* is heat stable and

non-volatile in tap water, is not extractable by cold ether or alcohol (both cold and boiling), but is readily adsorbed and held by soil and charcoal particles and is inactivated by dilute H_2SO_4 solutions.

Summary

Poor germination of the seed of *Pennisetum ciliare* is due partly to the secretion of an inhibitor by the hull (bristles, glumes, sterile lemma, fertile lemma and palea) of the seed and partly to the impermeability of the seed coat to gases. Removal of the source of the inhibitor or adsorption of the inhibitor by soil or charcoal particles results in good germination. The inhibitor is evidently non-volatile in boiling water and is not extractable by cold ether and cold or boiling alcohol. It is not inactivated by these treatments, but it is inactivated by hot dilute H_2SO_4 solutions. Cutting of the gas-impervious seed coat results in increased germination. Acid-scarified fascicles germinate better than untreated fascicles because of the decreased source of the inhibitor as a result of scarification.

For field planting the fascicles may be scarified with concentrated sulphuric acid and dried before planting in order to obtain good germination. In the laboratory removal of the hull and cutting of the seed coat will result in maximum germination of this species.

Urochloa pullulans

Germination in soil

The seeds of this species used in the soil and petri dish series were obtained from seven different harvests from field-grown and greenhouse-grown plants. The "seed" is a spikelet composed of caryopsis, fertile lemma and palea, sterile lemma and palea, and glumes. At maturity the spikelet falls from the inflorescence with its parts intact. The percent normal seed ranged from 38 to 93.

In numerous preliminary trials in soil in which a large number of different treatments were involved, no treatment that produced an immediate increase in the germination of the fresh seed of this species was found. Nevertheless, it was soon discovered that prolonged storage at temperatures higher than room temperature hastened germination. It was also discovered that whereas dry storage at these temperatures hastened germination, wet storage was detrimental in that the seeds rotted. The effect of temperature on germination was thoroughly studied in the laboratory germination studies and will be discussed in detail in the petri dish series.

One lot of seed with the fertile lemma and palea removed (naked seed), germinated in soil after the fifth month of dry storage at room temperature. When germinated with the lemma and palea intact, the seed did not commence to germinate until after the tenth month of storage period. Another lot of seed which was stored dry at a temperature slightly higher than room temperature began to germinate after the ninth week of storage period when germinated with the lemma and palea removed.

Germination in petri dishes

Three different lots of seed harvested at different times were cured at room temperature for 2 to 4 weeks and then stored dry at various warm temperatures. Germination tests were conducted at intervals at room temperature, the seed being germinated naked and normal. The results of one lot are presented in table 25 (the other two lots produced similar results).

TABLE 25. Germination of *Urochloa pullulans* seed as affected by dry storage at warm temperatures.

10 caryopses for each treatment.
Germination in petri dishes at room temperature in one week.
Seed harvested July 1, 1943.
Storage started July 15, 1943.

Storage temperature	Seed germinated	Germination percentages after a storage period of —										
		0 weeks[1]	2 weeks	4 weeks	7 weeks	8 weeks	9 weeks	10 weeks	13 weeks	17 weeks	21 weeks	25 weeks
26.0°—30.4° C. Room temperature....	Naked	0	[2]0	[2]0	[2]0	[2]0	[2]0	[2]0	[2]0	[2 3]10	[2 3]10	[2 3]10
	Normal	0	0	0	0	0	0	0	0	0	0	0
24.1°—31.8° C. Greenhouse—shaded ..	Naked	0	[2]0	[2]0	[2]0	[2]0	[2]0	[2]0	[2]0	[2 3]40	[2 3]40	[2 3]40
	Normal	0	0	0	0	0	0	0	0	0	0	0
34.5° C. Germinator—dry	Naked	0	[2]0	[2 3]0	[2 3]0	[2 3]0	[2 3]10	[2 3]40	[2 3]70	[2 3]90	[2 3]90	[2 3]90
	Normal	0	[2]0	[2]0	[2]0	[2]0	[2]0	[2]0	[2]0	[2]0	[2]0	[2]0
39.2° C. Incubator	Naked	0	[2]0	[2 3]0	[2 3]0	[2 3]10	[2 3]20	[2 3]60	100	.....	.....	.....
	Normal	0	[2]0	[2]0	[2]0	[2]0	[2]0	[2]0	[2]0	[2]0	[2]0	[2 3]10
41.8° C. Incubator	Naked	0	[2]0	[2 3]0	[2 3]20	[2 3]30	[2 3]30	[2 3]30	[2 3]80	100	.....	.....
	Normal	0	[2]0	[2]0	[2]0	[2]0	[2]0	[2]0	[2]0	[2 3]10	[2 3]20	[2 3]30
44.9° C. Oven	Naked	0	[2]0	[2 3]0	[2 3]10	[2 3]10	[2 3]20	[2 3]20	[2 3]80	[2 3]80	[2 3]80	[2 3]80
	Normal	0	[2]0	[2]0	[2]0	[2]0	[2]0	[2]0	[2]0	[2]0	[2]0	[2 3]20

[1] Initial germination at time of storage.
[2] Roots of ungerminated seeds developed.
[3] Plumule of ungerminated seeds slightly extended.

From table 25 it is seen that dry storage at warm temperatures is very effective in hastening the germination of *Urochloa pullulans* seed. When germinated naked, most of the seeds stored at the four highest temperatures germinated after the thirteenth week of storage period, whereas the seeds stored at room temperature and at greenhouse temperature did not germinate. It was not until the end of the seventeenth week of the storage period that the seeds stored at the low temperatures started to germinate. The higher the temperature, the earlier the initiation of germination, but 39.2° C. seems to be the optimum temperature, the seeds stored at this temperature being the first to show 100 percent germination.

When germinated as normal seed, no germination occurred until the end of the seventeenth week of the storage period, and this occurred only in the lot stored at 41.8° C. Unlike the naked seed which rapidly increased in germination as the storage period lengthened, the normal seed increased in germination very slowly after the initial germination. Eight weeks after the initial germination of 10 percent, the lot stored at 41.8° C. germinated only 30 percent. By this time the lots stored at 39.2° and 44.9° C. had also started to germinate.

Due to the exhaustion of seed supply this experiment unfortunately was concluded after 25 weeks of storage. Nevertheless, the following general relationship in regard to the effect of storage temperature on the germination of this species may be drawn from this and the preceding experiments: When germinated as normal seed, the seed requires a storage period of about 10 months at room temperature before it begins to germinate. The same lot of seed when germinated with the lemma and palea removed requires about 4.5 months for the initial germination. A storage period of about four months at a warm temperature (41.8° C.) is required for the normal seed to commence germinating. When germinated with the lemma and palea removed the seed stored at the warm temperatures (34.5° to 44.9° C.) requires approximately two months of storage for initial germination.

In another series seeds were stored dry at higher temperatures of 54.7° and 61.9° C. At 54.7° C. no germination occurred even after 15 weeks of storage. It was found that 61.9° C. was definitely detrimental to germination in that after the seventh week of storage period the seeds rotted when subjected to conditions favorable for germination.

The effect of alternating temperature was also studied. Some seeds were stored dry at 24-hour alternations between 44.9° C. and room temperature. The results showed that this treatment was only about as effective in breaking dormancy as the constant 44.9° C. storage.

A peculiar characteristic, not reported in the literature for other grass seeds, was observed in this species under germination conditions. This characteristic is the elongation of the radicle with the resulting production of young roots and the slight elongation of the plumule but without production of a normal green shoot. Such a seed was not considered as being germinated, because a germinating seed produces a green shoot in 2 to 3 days. Furthermore, a seed in a germination medium with roots and plumule extended but with no green shoot may remain in this condition for an extended period until attacked by rot organisms. It is seen from table 25 that the effect of storage is that the roots develop first followed by the elongation of the plumule followed by the development of the green shoot (normal germination) as the storage period lengthens.

When germinated naked the roots develop at about the same time regardless of storage temperature, but the plumule elongates earlier when the seed is stored at the higher temperatures than when stored at the lower temperatures. This, of course, accounts for the early germination of the seeds stored at the warm temperatures.

When germinated as normal seed, the roots of the lot stored at the two lowest temperatures did not develop, but those of the lots stored at the higher temperatures developed almost immediately after storage. The plumule was not extended until the end of the seventeenth week of storage (at 41.8° C. only) when the normal seed started to germinate.

Since the removal of the lemma and palea (naked seed) resulted in an early germination of the seeds stored at warm temperatures, attempts were made to determine the role these structures were playing in preventing germination. The sterile lemma and palea and the glumes were disregarded in these studies, because it is not conceivable that these loosely attached structures play any part in hindering germination. These structures, of course, must be removed before any treatment to the fertile lemma and palea can be applied. Seeds of a lot stored at 39.2° C. for 16 weeks and known to germinate well when germinated naked were variously treated and germinated at room temperature. The treatments and germination results are recorded in table 26.

TABLE 26. Role of lemma and palea on the germination of *Urochloa pullulans* seed.

Seed stored dry at 39.2° C. for 16 weeks.
10 caryopses for each treatment.
Germination in petri dishes at room temperature for one week.

TREATMENT	GERMINATION
	Percent
Lemma and palea removed	[1]90
Lemma and palea removed and seedcoat cut	70
Lemma and palea punctured	10
Only lemma removed	30
Only palea removed	30
Lemma and palea intact	[2]0

[1] Also 90 percent germination in soil.
[2] Also 0 percent germination in soil.

As in the case of the seed of *Paspalum notatum,* the presence of the fertile lemma and palea around the caryopsis of *Urochloa pullulans* resulted in a reduction in germination (table 26). The removal of only the lemma or of only the palea was not sufficient to increase the germination. Puncturing the lemma and palea was also ineffective. Injury to the seed which occurred when the seed coat was cut resulted in the lower germination of this seed as compared with the germination of the uncut naked seed. Thus mechanical scarifiers and other means which modify the nature of the seed coat were found to be impractical for removing the lemma and palea from this seed.

In several other similar experiments, results which are in agreement with those presented in table 26 were obtained. In one experiment the removal of only the lemma was slightly more effective than the removal of only the palea in increasing germination. That the structures enclosing the caryopsis were not producing a substance that inhibited germination was shown in another experiment.

To determine if water absorption was the limiting factor in the low germination of this species, seeds were subjected to the treatments given in table 26 and soaked in tap water at room temperature. After a soaking period of 24

hours, the seeds were examined under a microscope. In every treatment the caryopsis absorbed water, but there was a difference in the degree of swelling and in the development of the plumule and radicle. With the lemma and palea removed (and seed coat cut) the caryopsis was uniformly swollen to maximum expansion with the plumule and radicle already projecting. With only the lemma removed, the dorsal side of the caryopsis was swollen to maximum expansion, but the ventral side was only slightly swollen with the plumule and radicle starting to project. With only the palea removed the ventral side was swollen to maximum expansion, but the dorsal side was only slightly swollen with the plumule and radicle not yet projecting. With the lemma and palea intact or punctured, the caryopsis was only slightly swollen with the plumule and radicle not protruding. This study together with the results presented in table 26 also indicated that the lemma and palea and seed coat did not interfere with the passage of gases.

From the above considerations of the germination studies made on the seed of *Urochloa pullulans,* it seems that dormancy of this species is caused first, by a need of some physiological change within the seed and, second, by the presence of tough enveloping structures around the caryopsis. The physiological change within the seed is known as after-ripening, and the tough enveloping structures are the lemma and palea.

As far as is known to the author, this is the first reported case of a grass seed requiring a prolonged after-ripening period for germination. This also seems to be the first instance in which warm temperatures have been found effective in breaking the dormancy of seeds. After-ripening is usually effected by subjecting moist seeds to low temperatures for a prolonged period—so-called stratification treatments (1, 4, 11, 14, 18, 22, 23, 25)—but in the present instance, low temperatures were ineffective whether the seed was treated dry or moist. The exact nature of the effect of warm temperatures on the after-ripening process is not known, but one effect of warm temperatures is the expected reduction in the moisture content of the seed. In one case the moisture content of the seed stored at 39.2° C. and at room temperature for 18 weeks was respectively, 11.2 percent and 15.5 percent.

The effect of the fertile lemma and palea on the germination of the seed of this species is similar to the effect of these structures on the germination of the seed of *Paspalum notatum.* These structures prevent the maximum expansion of the caryopsis and thus prevent germination. They do not, however, interfere with moisture absorption or with the passage of gases.

Summary

Delay in germination of the seed of *Urochloa pullulans* is caused by a state of dormancy within the seed and by a tough lemma and palea which prevent maximum expansion of the caryopsis. Dormancy within the seed is overcome by after-ripening the dry seed at warm temperatures (34.5° to 44.9° C.) for approximately 13 weeks. This treatment followed by the removal of the lemma and palea results in an early maximum germination.

Dry storage at warm temperatures for a prolonged period before planting in the field will help to hasten and increase the germination of this seed. If the hull is removed after the storage period, a shorter period of storage will be required, but there are no practical means for removing the hull without injuring the embryo. For laboratory germination the hull can be removed by hand.

DISCUSSION AND CONCLUSION

According to Crocker (13), dormancy, or the inability of seeds to germinate, is caused by any of the following conditions of the seed:

1. Rudimentary embryos that must mature before germination can begin;
2. Complete inhibition of water absorption;
3. Mechanical resistance to the expansion of the embryo and seed contents by enclosing structures;
4. Encasing structures interfering with oxygen absorption by the embryo and perhaps carbon dioxide elimination from it, resulting in the limitation of the processes dependent upon these;
5. A state of dormancy in the embryo itself or some organ of it, in consequence of which it is unable to grow when naked and supplied with all ordinary germinative conditions;
6. Combination of two or more of these;
7. Assumption of secondary dormancy.

The cause of delayed germination of the seed of *Sporobolus wrightii* probably falls in classification 4 of the above causes of dormancy, since soaking the seed in water, cutting the seed coat, or use of alternating temperatures improves germination. It has been shown that absorption of water by the seed is not a factor in the delayed germination of this species, and it is very likely that these treatments influence the gas exchange, an idea which is in line with what Toole (58, 61) found as to the possible effect of scarification on gas exchange and with what others (12, 33) found as to the effect of temperature on gas exchange. The exact role of alternating temperatures has not been determined.

Judging by the increased germination produced by alternating temperatures and by cutting the seed coat under unfavorable temperature conditions, the cause of delayed germination in the seed of *Sporobolus airoides* may also be in classification 4. The further increase in germination due to KNO_3, however, and the favorable effect of vitamin C may put this seed under some category not listed by Crocker (13) who states that little or no salt absorption takes place through the dead semipermeable membrane of the seed. Later investigators (44, 45, 3, 40, 42) have shown that salts are absorbed in varying degrees. In the present investigations, in all instances where KNO_3 was used as a stimulant, the salt was effective even after washing or drying the treated seed, indicating a strong possibility that the salt was absorbed by the seeds. Niethammer (39) classifies stimulation of germination by chemicals into three groups; namely, primary stimulation in which the chemical enters the seed and acts on its protoplasm; secondary stimulation in which the chemical modifies the permeability of the seed coat; and apparent stimulation in which the chemical sterilizes or stimulates without entering the seed. Although the specific role of KNO_3 is not known, it is believed that its stimulation is of the primary type and is possibly performing in a nutritive capacity. The salt does not increase the permeability of the seed coat to water.

The stimulation action series affecting germination of the seed of *Sesamum indicum* was found to be in the following order (55): for the anions,

$NO_3>Cl>SO_4>PO_4$; for the cations, $NH_4>Na>K>Ca$. Ammonium salts were more effective than nitrates. KNO_3 has been found effective in stimulating the germination of seeds of grasses (2, 36, 58, 60, 56, 57). Other forms of nitrates have also been used successfully (36, 19, 20).

Ascorbic acid (vitamin C) in low concentrations (17) has been found to stimulate germination as it did with *Sporobolus airoides* seed. Vitamin C is a good reducing agent and its beneficial effect on germination may have been the result of reduction of the carbohydrate molecule in the respiration process. Thus it seems that the delayed germination in the seed of *Sporobolus airoides* is due to an external (seed coat characteristic) cause and some internal (nutritive and respiratory) cause.

What has been said as to the possible roles of alternating temperatures and KNO_3 on the germination of the seed of *Sporobolus airoides* holds for the seed of *Poa pratensis* and of *Cynodon dactylon.* The favorable effect of reduced oxygen pressure in the germination of the seed of *Cynodon dactylon,* as found in the present study in a small experiment[5] and also in an earlier study by Morinaga (37), is not understood. Takahashi (52) found that germination of rice seed was possible in the absence of oxygen. Later Jones (29) found that reduced oxygen pressure was detrimental to rice germination, and Edwards (21) found that the seed of *Peltandra virginica* germinated in almost complete absence of oxygen, the coleoptile increasing its original length two to three times by the elongation of the cells already developed in the embryo.

The causes of delayed germination in the seed of *Pennisetum setosum* seem to be the same as for the seed of *Sporobolus airoides.* First, its response to alternating temperatures and to cutting of the seed coat place it in classification 4 of Crocker (13). These treatments modify the seed coat and facilitate gas permeability. In the second place, it also responds to KNO_3 and vitamin C. A much greater response to NH_4SCN was obtained than with KNO_3 or vitamin C. Although Thompson and Kosar (53, 54) and Gemeinhart (24) found thiocyanates to be beneficial to seed germination, Brun (6) observed that dilute solutions of NaSCN inhibited germination. Cyanides are known to affect respiration favorably, and sulphur in the thiocyanate is an excellent oxidation-reduction catalyst. Further evidence of the function of NH_4SCN in respiration is the susceptibility of the treatment to temperature differences as shown by the germination results. The delayed germination in *Pennisetum setosum* seed seems to be due to seed coat characteristics and the need for some respiratory and perhaps nutritive stimulation as in the seed of *Sporobolus airoides.*

In the seed of *Panicum prolutum,* delayed germination is wholly due to the character of the seed coat which prevents oxygen absorption and CO_2 elimination (Crocker's cause of dormancy 4). Any treatment (acid scarification, cutting of seed coat, soaking in water or extreme alternating temperatures) that modifies the gas-impervious seed coat improves germination, whereas salts have no effect. Normal seed absorbs water freely. In the germination of poverty grass, delay in germination was believed to be the result of the impervious nature of the seed coat to gas exchange (58). Cocklebur seed coats exclude oxygen supply and thus hinder germination (12).

Since delayed germination in the seed of *Cenchrus biflorus* seems to be due to the nature of the seed coat which hinders normal gas exchange, this seed

[5] Experimental data not reported in this bulletin.

also belongs in classification 4 of Crocker's causes of dormancy. As in the case of *Panicum prolutum* seed, mechanical scarification which alters the gas-impervious seed coat promotes germination, and the normal seed freely absorbs water.

Crocker's third cause of dormancy listed above (mechanical resistance to the expansion of the seed by enclosing structures) is the logical one to explain delayed germination of the seed of *Paspalum notatum.* With the seed of *Alisma plantago,* Crocker and Davis (15) found that dormancy was due to mechanical restraint of the seed coat to the complete swelling of the embryo which exerted a pressure of about 100 atmospheres against the seed coat. Unlike this seed in structure but similar in the type of dormancy, the seed of *Paspalum notatum* was delayed in germination by the tough lemma and palea and not by the seed coat. The lemma and palea are so tough that any part of them remaining on the caryopsis materially reduces the maximum imbibitional swelling of the embryo and of the caryopsis in general, thereby preventing germination. If the germ starts growth, it is trapped within the hull and never emerges. Any treatment which tends to free the caryopsis from the enveloping hull promotes germination. Caryopses without hulls readily absorb water to their imbibitional capacity.

The delay in germination of the seed of *Pennisetum ciliare* is due in part to Crocker's fourth cause of dormancy and in part to the production of an inhibitor by the hull of the seed. The caryopses readily absorb water. Removal of the source of the inhibitor improves germination, and cutting the seed coat of the ungerminated, fully swollen seed also promotes further germination as a result of increased gas exchange through the seed coat. The possibility of the inhibitor being free ammonia as was found by Stout and Tolman (50, 51) to be the case in germinating sugar beet balls does not seem to obtain here, since boiling the hulls in tap water or washing the fascicles in running water does not alter the inhibitory effect. The inhibitor cannot be inactivated or extracted with hot or cold alcohol, or with cold ether; but it can be inactivated with hot dilute H_2SO_4 solutions and is readily adsorbed on colloidal surfaces such as occur on soil and charcoal particles. The production of HCN by germinating seeds of some Prunaceae and Pomaceae is said to interfere with germination (31), but tests for cyanide in the hulls of *Pennisetum ciliare* seed gave negative results.

The germination of the seed of *Urochloa pullulans* has been shown to be delayed by two factors: First, a condition of dormancy within the seed (probably in the embryo) and second, the presence of enclosing structures which prevent maximum expansion of embryo and caryopsis in general. Therefore this is a case of dormancy caused by conditions number 5 and 3 of Crocker. The first condition is corrected by subjecting the dry seed to warm temperatures for a prolonged period, and the second condition is corrected by removing the tough lemma and palea from the caryopsis. This seed apparently after-ripens at warm temperatures, whereas other seeds after-ripen at cold temperatures (1, 4, 11, 14, 18, 22, 23, 25). What effect the drying at warm temperatures has on the after-ripening process is not known. The presence of the lemma and palea in this seed has the same effect on germination as the presence of these structures in the seed of *Paspalum notatum.* In order to effect early germination of *Urochloa pullulans,* seed must first be after-ripened at a warm temperature and then the lemma and palea must be removed from the caryopsis.

Of the several causes of dormancy listed by Crocker (13), four apply to the grass seeds studied here: Mechanical resistance of enclosing structures which prevents maximum expansion of the seed; prevention of gas exchange by the character of the encasing structure; dormancy in the embryo itself; and combinations of these causes (Crocker's causes of dormancy, numbers 3, 4, 5, and 6 respectively). To these four may be added two more causes of dormancy in grass seeds: Lack of a stimulant to hasten the respiratory and perhaps nutritive activity within the seed, and production of an inhibitor by the hull of the seed.

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